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TITLE:

Minority Undergraduate Research in Prostate Cancer: Bridging
Opportunities for Postbaccalaureate Education

PRINCIPAL INVESTIGATOR:

Robert A. Sikes, Ph.D.

CONTRACTING ORGANIZATION:

University of Delaware
Newark, DE 19716

REPORT DATE:

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PREPARED FOR: U.S. Army Medical Research and Materiel Command
               Fort Detrick, Maryland 21702-5012

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Newark, DE 19716

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13. SUPPLEMENTARY NOTES

14. ABSTRACT
Four students, 3 from Delaware State University and 1 from Lincoln University, were recruited to participate in prostate cancer research in laboratories at the University of Delaware. In compliance with the aims of our grant the students each received intensive research training over the 10-week summer program. All students were required to participate in enrichment activities that spanned the scope of intellectual property, careers in medicine and science, as well as good research practice. Also in compliance with our aims, this grant sponsored three Health Disparity round table discussions that covered a range of issues in minority health. These discussions included topics of access, economic status, racial profiling, provider perceptions/misconceptions and race-based medicine. All discussions were based on primary literature as a lead in to the topic followed by group discussions. All students presented posters in a research symposium with over 500 participants from UD, Wesley, UMBC and other regional schools. Selected students participated in regional competitions.

15. SUBJECT TERMS
Undergraduate students, minority, abstracts, awards

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Introduction:

Due to the extremely low levels of minority faculty and graduate students in the sciences, the DoD Majority Institution (MI) /Historically Black College and University (HBCU) program was intended to foster and promote the interest of minority students in basic science and research by partnering one or more HBCU with a sponsoring MI. In Delaware, this has been accomplished by coordinating student recruitment from Delaware State University and Lincoln University to perform funded summer research in prostate cancer laboratories at the University of Delaware. Our Aims were to 1) offer a 10-week summer research program to five qualified minority students, 2) Offer a summer enrichment program to these students and 3) offer activities and extended research at the participating HBCUs during the following academic year.

Body:

In compliance with Aim 1, and upon the recommendation of the faculty campus coordinators at Delaware State University (Dr. Cindy van Golen) and Lincoln University (Susan Safford), 3 students from DSU and 1 student from Lincoln University were chosen for admission into the University of Delaware's training program in Prostate Cancer after being interviewed by prospective faculty mentors at UD. As noted previously, our HBCU facilitators have been having an increasingly difficult time with this task due to high demand, or competition from other funded summer programs recruiting minority students nationally. In addition, the precipitous drop in mentor funding has reduced our available mentor pool. Unfortunately, training programs do not realize that solid research and close supervision occurs in small labs where a student having money to help defray the cost of research is appreciated greatly.

Table 1. Prostate Cancer Research Scholars FY2011

Student	School	UD Mentor	Project
Talearia Young	DSU	Randall Duncan	The Expression of TRPV in Prostate Cancer Cell Lines C4-2 and LNCaPs, with Treatment of IGF and TRPV Inhibitors
Brezelle Bey	LU	John Koh	Intein-based Expression and Labeling of the Androgen Receptor Ligand Binding Domain
Brenda Mogere	DSU	Robert Sikes	Effects of ATP and Adenosine on a Metastatic Prostate Cancer Cell Line (C4-2B4)
Yanique Conie	DSU	Ken van Golen	Determining the Sensitivity of IBC cells to Imatinib Using ATP-CRA Assay

In compliance with Aim 2, students attended weekly seminars related to research (Appendix 1). In addition our students attended discussion three additional sessions on the topic of *Healthcare Disparities*. Prior to each session students were assigned to read both popular and scientific literature regarding the genetic, socio-economic or medical causes of healthcare bias. UD faculty from the Departments of Biological Sciences, Chemistry and Biochemistry moderated the discussions.

Key Research Accomplishments:
The students in this program were expected to make significant progress in research over a 10-week period. Students instructing them in laboratory procedures that included basic liquid handling, safety, and use of technology and equipment required. Despite this, the amount of publishable data that each student collected during this short time is amazing. Additionally, students were instructed to journal their research experience to enhance their level of comfort of communicating what skills and techniques they learned as well as understanding the research project. At the end of the summer program, each student presented the results of their research at the University of Delaware's undergraduate research symposium and celebration (See Appendix 2), which required the students to produce a written abstract and poster for presentation (see abstract numbers 42, 70, 77, 104 in Appendix 2, Abstracts are listed in Appendix 6). The symposium was modeled after the Experimental Biology meeting, where posters and talks occurred simultaneously and where there was a plenary lecture by a senior investigator of national prominence. In addition, student research from dedicated students, those who did more than one summer or continued through the fall, paid off with peer-reviewed publications (Appendices 3-5, covering 4 DoD HBCU Students: Navpreet Tung, Mishiriki Jenkins, Jhoneil Cooper, and Alfayo Michira).

Reportable Outcomes Year 3:
4 posters and 3 manuscripts.

Conclusions:
Our students frequently state that their summer experience has made them evaluate research as a career option. In many cases this has resulted in graduate school applications instead of vocational programs in Nursing or other health related field. For example, Navpreet Tung, now applying to graduate schools, and Wachen Peters, now enrolled in UD Biological Sciences Doctoral Program. Our students get hands on research, not observation, and leave excited. We have had many students who apply for a second year. This typically results in additional papers, meetings or posters and encourages their application to professional schools. This fact alone suggests that we have a viable, rewarding program that is not redundant or repetitious from year to year. We are producing students of quality from HBCUs who can compete regionally and win prizes in poster competitions based on their bench results.

References:
None

Appendices:
1. Enrichment program schedule (Summer 2011)
2. UD Undergraduate Research Symposium-program
3. Miles, Tung et al, Prostate 2012
4. Chatterjee et al, J. Signal transduction 2011
5. Pan et al, Novel antiandrogens (Draft)
6. Student Proposals or Summary for Summer Research
7. Photos of students at posters during symposium

Appendix 1—Summer Enrichment Program 2011

DATE	PROGRAM
On-line On your own	*What do you need to know about Safety in the Research Laboratory?* *Please note: You need to have completed safety instructions in your research laboratory and/or on-line before you start work in a laboratory. If you have questions, contact Occupational Health and Safety.*
Thurs June 9 4:00-5:30 Willard Hall	*Undergraduate Research Ethics Conference* **Dr. Harold White** (Dept. Chemistry & Biochemistry, Director UD's HHMI Undergraduate Science Education Program), **Dr. Thomas Powers**, Department of Philosophy and co-director of the **Science, Ethics, and Public Policy program** administered by the Delaware Biotechnology Institute, and graduate students. Willard Hall Building, rooms 109, 116, 135, 208, & 215
June 15	*What are you doing here this summer? Introduction to Research and issues you may encounter.* **Dr. David Usher** (Dept. of Biological Sciences, Assoc. Dir. UD's HHMI Undergraduate Science Education Program)
June 22	*Student Voices. Students who have done undergraduate research for more than a year describe their experiences.* **Michael Brister,** Soma Jobbagy, and **Erica Boetefuer**. **All three presented research posters at the 2011 Experimental Biology Meetings in Washington, D.C.** **Dr. Justin DiAngelo** BS Biology '02, PhD UPenn '10 (Asst. Prof. Cell Biology at Hofstra University.)
June 29	*Don't stop now-Other University opportunities?* **Susan Serra,** Service Learning Coordinator and **Katharine Kerrane,** Senior Associate Director, Honors Program, and undergraduate panelists discussing National and International Scholarship Opportunities, Semester Abroad, Service Learning, and related opportunities. (Goldwater, Marshall, Mitchell, Rhodes, and Truman Scholarships, Fulbright Fellowships)
July 6	*How are things going? Mid-Session Perspectives.* **Dr. David Usher** (Dept. of Biological Sciences, Assoc. Dir. UD's HHMI Undergraduate Science Education Program), **Dr. Harold White** (Dept. Chemistry & Biochemistry, Director UD's HHMI Undergraduate Science Education Program). **Dr. Gary Laverty** (Dept. of Biological Sciences).
July 13	*How do I get into Graduate School? (Must attend this session and/or the August 3 session)*

	Dr. David Usher, (Dept. of Biological Sciences), **Dr. Melinda Duncan**, (Dept. of Biological Sciences), **Dr. John Pelesko**, (Dept. of Mathematical Sciences) **Dr. Svilen Bobev**, (Department of Chemistry and Biochemistry) **What is it like to be a graduate student in the sciences?**
July 20	***Managing a career in science.What is it like to be a scientist in academia or industry?*** ***Career biographies from academic and industrial scientists.*** **Dr. Patricia DeLeon**, Trustees Distinguished Professor, Biological Sciences **Dr. Jill Higginson**, Director Center for Biomedical Engineering Research **Dr. Yli Remo Vallejo**, Vice President, R&D, ANP Technologies, Inc. **Dr. Satish Sharma**, Director R & D, Fraunhofer Center for Molecular Biotechnology **Dr. Brian Pryor**, CEO, LiteCure **Dr. Janet Reed**, Partner-Intellectual Property, Potter Anderson & Corroon LLP Jeffrey Safran, Ph.D. and Travis Bliss, Ph.D., Associates at Potter Anderson
July 27	***How to communicate your Results - Conferences (Talks and Posters)*** **Michael Brister,** BS Biochemistry**, Soma Jobbagy**, BS Biochemistry, and **Erica Boetefuer**, Biological Sciences **Judging Rubrics** for the ASBMB Undergraduate Poster Competition 2007 A good site for **instructions on poster preparation**. Another good site.
August 3	◇***How do I get into Medical or other professional Schools?*** ***(Must attend this session and/or the July 13 session)*** **Dr. David Usher** (Dept. of Biological Sciences), **Christine Arenson, MD** BS Chemistry '86, Director, Division of Geriatric Medicine, Jefferson Medical School. **Faith A. Brosch, MD** BS Biology '83, **Bruce Bowman MD, PhD**, **Soma Jobbagy**, BS Biochemistry

UNIVERSITY OF DELAWARE.

August 10, 2011

Dear Friends of Undergraduate Research and Service:

Welcome to the Second Annual Undergraduate Research and Service Celebratory Symposium. There is a saying "You can't judge a book by its cover". However, in this case – you can. As you see on the cover of our program booklet, research and service at the University of Delaware is deeply informed by both disciplinary and interdisciplinary standards, and provides students with unique opportunities to synthesize their learning in new and creative ways. Within the last ten weeks, through the guidance of dedicated faculty a transformation has taken place: undergraduates involved in the summer scholar programs have transitioned from students to scholars. They have worked side by side with University of Delaware faculty and—in some cases, with community partners—in labs, studios, libraries, schools, hospitals and some international settings. Today you will observe the result of this effort.

New this year is an **Interdisciplinary Undergraduate Research in Sustainability Prize** established by the University of Delaware Undergraduate Research Program, the University Library, and Gale, a leading organization in e-research and educational publishing, to encourage undergraduate research and projects in the area of sustainability. This prize is in conjunction with the implementation of GREENR (Global Reference on the Environment, Energy, and Natural Resources). The prize winners will be announced during the closing program.

This program would not be possible without the vision and commitment of the members of the *Alliance of Summer Scholar Programs*. This group is comprised of directors, coordinators, and representatives of each of the University of Delaware Summer Scholar Programs. This event is a result of the collaborative efforts of this group.

On behalf of the Alliance of Summer Scholar Programs, thank you for attending this program. We hope you leave with new ideas and strategies for future interdisciplinary and collaborative research, service programs, and projects, and have a deeper appreciation for the intellectual and creative potential of undergraduate students at the University of Delaware.

Sincerely,

Lynnette Young Overby

Lynnette Young Overby, Ph.D.
Professor, Department of Theatre & Faculty Director
Undergraduate Research and Experiential Learning

Office of the President

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August 10, 2011

Dear Colleagues and Friends:

Welcome to the University of Delaware's Second Annual Undergraduate Research and Service Celebratory Symposium. What started in 1980 with a few highly motivated students pursuing discrete projects became an exemplary summer scholars program of many discrete programs. Now with the structural support of the Alliance of Summer Scholars Programs and the financial support of the Office of the Provost, undergraduate student scholars participate in one integrated event.

As you'll see today, undergraduate students are engaged in important work representing every discipline. There are many winners in this enterprise, from the faculty sponsor who gains an assistant for his or her research, to the community partner who receives a wealth of knowledge and service, to the undergraduate student who is forever changed by the independent thinking and problem solving experience.

I congratulate everyone involved in making University of Delaware Undergraduate Research and Service such a special source for hands-on, minds-on scholarship, and for preparing students for exciting and rigorous careers.

Sincerely,

Patrick T. Harker
President

Undergraduate Research and Service Celebratory Symposium
Clayton Hall Conference Center
Wednesday, August 10, 2011
8:30 a.m. - 4:30 p.m.

8:00 – 8:45   Registration
                Breakfast . . . . Lobby
                Poster Set-Up . . . . Room 101 A/B

8:40 – 8:55   Welcome Remarks, Provost Tom Apple . . . . Lobby

9:00 – 10:30   *Poster Session I* (Even numbered & INBRE students stand by poster) . . . . Room 101 A/B

9:00 – 9:50   *Oral Session 1*
                1. Children and the Arts . . . . Room 121   pg. 18
                2. Health and Policy . . . . Room 122   pg. 18
                3. Literature . . . . Room 123   pg. 19
                4. Exercise and Health . . . . Room 124   pg. 19

10:00 – 10:50   *Oral Session 2*
                1. Music and Human Behavior . . . . Room 121   pg. 19
                2. International Relations . . . . Room 122   pg. 19
                3. Teacher Education . . . . Room 123   pg. 19
                4. Historical and Personal Economics . . . . Room 124   pg. 19

10:45 – 12:15   *Poster Session II* (Odd numbered students stand by poster) . . . . Room 101 A/B

11:00 – Noon   *Oral Session 3*
                1. Art/Dance/Journalism . . . . Room 121   pg. 20
                2. Music and Art Therapy/Art Conservation . . . . Room 122   pg. 20
                3. Politics/Public Policy . . . . Room 123   pg. 20
                4. Movement and Cognition . . . . Room 124   pg. 20

12:00 – 1:20   LUNCH . . . . Pencader 106, 115A&B;
                                                                                                                  Clayton 119 & 120

1:30 – 2:30   HHMI Keynote Speaker Graham Hatfull . . . . Auditorium Room 128
                Professor of Chemistry, Massachusettes Institute of Technology
                "Exploration and Exploitation of Mycobateriophages"

 1:30 – 2:30   *Oral Session 4*
                1. History . . . . Room 121   pg. 21
                2. Crime and Rehabilitation . . . . Room 122   pg. 21
                3. Consumer Behavior and Environmental Studies . . . . Room 123   pg. 21
                4. Exercise and the Elderly . . . . Room 124   pg. 21

2:40 – 3:30   *Oral Session 5*
                1. Science and Engineering . . . . Room 125   pg. 22
                2. Language and Media . . . . Room 121   pg. 22
                3. Law . . . . Room 122   pg. 22
                4. Anthropology . . . . Room 123   pg. 22
                5. Social and Educational Support Systems . . . . Room 124   pg. 22

3:40 – 4:00   UD Creamery Ice Cream, courtesy of the College of Agriculture and Natural Resources

4:00 – 4:30   Closing Remarks: Deputy Provost Nancy Brickhouse . . . . Auditorium, Room128

                Announcement of Interdisciplinary Undergraduate Research in Sustainability Prize
                Raffle with giveaway prizes

4:30-6:30   Art Show – all attendees are invited . . . . Studio Art Building

LIFE SCIENCES – (Agriculture and Natural Resources, Food and Resource Economics, Animal and Food Sciences, Entomology and Wildlife Ecology, Plant and Soil Sciences, Biotechnology, Biological Sciences, Neuroscience)

AGRICULTURE AND NATURAL RESOURCES

1) Sarah Ackerman, Agriculture and Natural Resources (McNair)
Gerald Kauffman, Institute for Public Administration
Restoration of Shad and Anadromous Fish in the White Clay Creek

2) Brandon Bruce, Agriculture and Natural Resources (EPSCoR) (DSU)
Christopher Heckscher, DSU - Agriculture and Natural Resources
The Effect of Ambient Temperature on the Incubation Behavior of the Female Veery

3) Amy Cannon, Agriculture and Natural Resources (EPSCoR) (DSU)
Gulnihal Ozbay, DSU - Agriculture and Natural Resources
Monitoring Aquatic Health in Wastewater Discharge Point Source in Delaware Inland Bays Tidal Canal: a Case Study on Heavy Metal Contaminants

4) Andrew Kluge, Agriculture and Natural Resources (EPSCoR) (DSU)
Gulnihal Ozbay, DSU - Agriculture and Natural Resources
Determining Easter Oyster (Crassostrea virginica) Recruitment on Cultch for the Delaware Inland Bays Oyster Gardening Project

5) Victoria Fitchett, Agriculture and Natural Resources (EPSCoR) (DSU)
Theresa Szabo-Maas, DSU - Agriculture and Natural Resources
Air versus Water Incubation and the Effects of 17β-Estradiol on Fundulus Heterocitus Embryonic Development

FOOD AND RESOURCE ECONOMICS

6) Ryan O'Dowd, Computer Science (EPSCoR)
Kent Messer, Food and Resource Economics
An Economic Analysis of the Visual Effects of Offshore Wind Projects

ANIMAL AND FOOD SCIENCES

7) Stephanie Chew, Animal and Food Sciences (CANR)
Robert Dyer, Animal and Food Sciences
Elevated Pro-Inflammatory Cytokines and Metalloprotease Gene Transcription in Early and Late Bovine Sole Ulceration

8) Monica Sterk, Pre-Veterinary Medicine (S&E)
Robert Dyer, Animal and Food Sciences
Establishing an In Vitro Culture Model for Rumen Epithelial Cells

9) Stephanie Shapiro, Pre-Veterinary Medicine (S&E)
Carl Schmidt and Robert Dyer, Animal and Food Sciences
In Vitro Culture Models for Avian Intestinal Enterocytes

10) Noelle Diana, Pre-Veterinary Medicine (S&E)
Tanya Gressley, Animal and Food Sciences
The Effect of Thymol on Staph aureus Killing by Bovine Neutrophils

11) Emily Mackey, Pre-Veterinary Medicine (S&E)
Tanya Gressley, Animal and Food Sciences
Measurement of Starter Intake and Growth Response of Dairy Calves Fed Traditional Calf Starter versus a Reduced Protein Amino Acid Balanced Calf Starter

12) Criztal Hernandez, Food Agribusiness Management (CANR)
Dallas Hoover and Kalmia Kniel, Animal and Food Sciences
Removal of Salmonella Newport and E. coli 0157 from Water Using a Zero Valent Iron Filtration System

13) Kayla Titus, Dietetics (S&E)
Larry Cogburn, Animal and Food Sciences
Differential Gene Expression in Hormonally-Induced Fat and Lean Phenotypes

14) Jennifer Wilkinson, Pre-Veterinary Medicine (S&E)
Mark Parcells, Animal and Food Sciences
Development of a Canine Coronavirus (CCoV) Vector System for the Treatment of Canine Hemangiosarcoma

15) Melissa Ehrich, Food Science (S&E)
Changqing Wu, Animal and Food Sciences
The Effects of Ozone, Mild Heat, Organic Acids and Thymol on the Quality of Green Onions and Blueberries

ENTOMOLOGY AND WILDLIFE ECOLOGY

16) Melissa Richard, Wildlife Conservation (S&E)
Douglas Tallamy, Entomology and Wildlife Ecology
Evaluating Food Web Complexity of Invaded Habitats

17) Jessica Ermak, Wildlife Conservation (S&E)
Christopher Williams, Entomology and Wildlife Ecology
Building a Predictive Habitat Model for Muskrat (Ondantra zibethicus) in New Jersey

18) Marissa Goldstein, Wildlife Conservation (S&E)
Christopher Williams, Entomology and Wildlife Ecology
A Comparison of Sampling Methodologies to Improve Carrying Capacity Estimates of American Black Ducks in New Jersey

19) Ashlee Schaeffer, Wildlife Conservation (S&E)
Charles Bartlett, Entomology and Wildlife Ecology
Diversity of Delphacid Planthoppers (Hemiptera: Fulgoroidea) of the Selenge River Basin, Mongolia

20) Kevin Schmidt, Environmental Science (EPSCoR)
Greg Shriver, Entomology and Wildlife Ecology
Neotropical Migratory Bird Survival in a Fragmented Landscape

21) Jeffrey Smith, Ecology/Environmental Science (S&E)
Judith Hough-Goldstein, Entomology and Wildlife Ecology
The Behavior of a Biological Control Agent

22) Connie Gatlin, Entomology (CANR) (Lincoln)
Judith Hough-Goldstein, Entomology and Wildlife Ecology
A Comparison of Arthropod Diversity in an Established Wetland vs. a Newly-created Wetland

PLANT AND SOIL SCIENCES

23) Kasia Dinkeloo, Plant Science (S&E)
Nicole Donofrio, Plant and Soil Sciences
The TmpL Homolog in the Rice Blast Fungus: a Gene Predicted in Sensing Reactive Oxygen Species

24) Matthew Fischel, Environmental Soil Science (EPSCoR)
Donald Sparks, Plant and Soil Sciences
Kinetics of Arsenite Oxidation by Manganese Oxide Minerals: Importance for Water Quality and Environmental Sustainability

25) Mara Hyatt, Biological Sciences (EPSCoR)
K. Eric Wommack, Plant and Soil Sciences
Uncovering Viral Diversity through Ribonucleotide Reductase

26) Helen Schmidt, Biochemistry (HHMI/EPSCoR)
K. Eric Wommack, Plant and Soil Sciences
Unexplored Diversity of Marine Viral DNA Polymerases Revealed through Metagenomics

27) Jeffrey Wray, Biological Sciences (EPSCoR)
K. Eric Wommack, Plant and Soil Sciences
Diamonds in the Rough: Finding Chaperonins in Viral Metagenome Data

28) Kamedra McNeil, Molecular Biology (CANR) (Winston-Salem)
Janine Sherrier, Plant and Soil Sciences
Early Development of Symbiotic Root Nodules in Pisum sativum

29) Janet Cordero, Biology (DSU) and Richard Muttai, Biotechnology (DTCC) (EPSCoR)
Harsh Bais, Plant and Soil Sciences
Beneficial Effects of Rhizospheric Microbiota on Rice

30) Sha-Phawn Williams, Biology (EPSCoR) (Lincoln)
Harsh Bais, Plant and Soil Sciences
Impact of Xenobiotics on Plants and their Below-ground Partners

31) Christopher Wright, Biochemistry (HHMI)
Pamela Green, Plant and Soil Sciences
Analysis of Small RNAs Associated with Plant Senescence

BIOTECHNOLOGY

32) Josh Vickers, Biotechnology (EPSCoR) (DTCC)
Kathryn Coyne, School of Marine Science and Policy
Diatom Analysis of Sediments in Blackbird Creek

33) Summer Thompson, Biotechnology (INBRE) (DTCC)
Jeffrey Caplan, Bio-Imaging Center
Examining Chloroplastic Stromule Interaction with Endoplasmic Reticulum and Nuclei during an Innate Immune Response

BIOLOGICAL SCIENCES

34) Dominick Harrison, Biology (INBRE) (DSU)
Thomas Bauer, Christiana Care-Graham Cancer Center
Measuring the Efficacy of Christiana Care Health System Multiple Disciplinary Disease Center Approach in Improving the Survival Rates of Stage 3 Lung Cancer in Delaware

35) Matthew Ralston, Biochemistry (UR)
Bruce Boman, Biological Sciences
Effect of Statin Drugs, via BMP Signalling, on Colon Cancer Cells

36) Hunter Witmer, Biology, (INBRE) (Haverford College)
Bruce Boman, Biological Sciences
Role of miRNAs in Regulating Colon Cancer Stem Cells

37) Carrie Barnum and Jennifer Sabatino, Genetics
Zohra Ali-Khan Catts, Cancer Genetics Program, Christiana Care Hospital
Importance of Banking Bio Specimens in High Risk Family Registries

38) Daniel Frailey, Biological Sciences (INBRE)
Kirk Czymmek, Biological Sciences
The Effect of Mid1 on Ca2+ Signatures and Polarized Cell Growth in the Fungal Genus Fusarium

39) Erin Bange, Biological Sciences (HHMI)
Patricia DeLeon, Biological Sciences
Characterization of Hyaluronidase3 in Human Sperm

40) Jocelyn Zajac, Biochemistry (HHMI)
Melinda Duncan, Biological Sciences
The Function of Sip1 in the Eye Lens

41) Victoria Roop, Biological Sciences (S&E)
Melinda Duncan, Biological Sciences
Characterization of the EMT Response in Crybb2Phil Mutants

42) Talearia Deshea Young, Biology (DoD) (DSU)
Randy Duncan, Biological Sciences
The Expression of TRPV in Prostate Cancer Cell Lines C42 and LNCaPs, with Treatment of IGF and TRPV Inhibitors

43) Olivia Hampton, Biology (INBRE) (Wesley)
Malcolm D'Souza, Wesley-Chemistry
Understanding Fragmentation of 0-p Tolyl Chlorothionoformate in Organic Solvents

44) Ashley Harmon, Biology (INBRE) (Wesley)
Malcolm D'Souza, Wesley-Chemistry
Evaluation of the Mechanism of Reaction of Crotyl Chloride

45) Jaci Knapp, Biology (INBRE) (Wesley)
Malcolm D'Souza, Wesley-Chemistry
Prevalence of Malignancy in Solitary Pulmonary Nodules of Patients Examined by the Thoracic Surgery Department at Christiana Care

46) Annie O'Connor, Biology (INBRE) (Wesley)
Malcolm D'Souza, Wesley-Chemistry
Use of Solvolysis to Understand Fragmentation of Synthetic Precursors

47) James Welsh, Biology (INBRE) (Wesley)
Malcolm D'Souza, Wesley-Chemistry
The Addition-Elimination Reaction of Pentafluorophenyl Chlorothionoformate

48) Ashley Shay, Biological Sciences (INBRE)
Vicky Funanage, Nemours-Biomedical Research
Hypoplastic Left Heart Syndrome: Molecular Consequences of Transcription Factor Mutations

49) Hamza Bhatti, Biological Sciences (HHMI/DE)
Deni Galileo, Biological Sciences
Time Lapse Analysis of Glioma Cell Behavior in Embryonic Brain Cell Co-Cultures

50) Ryan Hartman, Biological Sciences (Charles Peter White)
Deni Galileo, Biological Sciences
The Effect of Secreted L1 Ectodomain on Hydrocephalus in an Embryonic Chick Model

51) Mfon Ekanem, Biology (EPSCoR) (Lincoln)
Tom Hanson, School of Marine Science and Policy
Characterizing Isolates from an Arctic Tundra Soil Microbial Community

52) David Lipscomb, Biology (INBRE)
Melissa Harrington, DSU-Biological Sciences
Motor Neuron Development in Spinal Muscular Atrophy

53) Kari Cervelli, Biological Sciences (Charles Peter White)
Diane Herson, Biological Sciences
Mechanisms of Biocide Resistance and Observed Cross Resistance of Triclosan-Resistant Salmonella enterica serovar Typhimurium

54) Sarah Stamm, Biological Sciences (HHMI)
Diane Herson, Biological Sciences
Characteristics of Salmonella enterica with Reduced Susceptibility to Quaternary Ammonium Compounds

55) Dylan Lowe, Biological Sciences (S&E)
Catherine Kirn-Safran, Biological Sciences
Molecular Mechanisms Underlying Cartilage Defects in Perlecan Mutant Mice

56) Alex D'Angelo, Biological Sciences (HHMI)
Anja Nohe, Biological Sciences
Effects of CK2 Binding to BMPRIa on Receptor Localization Studied by ICCS

57) Lauren Gurski, Biological Sciences (S&E)
Anja Nohe, Biological Sciences
The Redirection of Adipocyte to Osteoblast through the Bone Morphogenetic Protein 2 Pathway with the Use of Casein Kinase 2

58) Matthew Liebross, Biological Sciences (Charles Peter White)
Anja Nohe, Biological Sciences
Synthesizing and Analyzing MicroCT Images of Cystein Capped Silver Nanoparticles with a 2nm Core

59) Adam Reese, Biological Sciences (Charles Peter White)
Anja Nohe, Biological Sciences
The Effect of Endoglin on Osteoblast Differentiation

60) Kevin Hutter, Chemical Engineering (UR)
Anja Nohe, Biological Sciences
A Physiologically-Based Pharmacokinetic Model of Bone Morphogenetic Protein 2 Distribution in Mice

61) Amber Griffin, Biological Sciences (UR)
Anja Nohe, Biological Sciences
Are Caveolae Smart?

62) Jessica Chopyk, Biological Sciences (EPSCoR)
Shawn Polson, Center for Bioinformatics and
Computational Biology
*Optimization of Methods for Enumeration and
Concentration of Environmental Viral Assemblages*

63) Samuel Widmayer, Biological Sciences (EPSCoR)
Shawn Polson, Center for Bioinformatics and
Computational Biology
*Exploring the Eastern Oyster Microbiome: Surveying
Bacterial and Viral Associates of Choptank River Populations*

64) Erica Boetefuer, Biological Sciences (HHMI)
Erica Selva, Biological Sciences
*The Role of atg18 in Signal Transduction Pathways during
Drosophila Development*

65) Allison McCague, Biological Sciences (S&E Biology)
Erica Selva, Biological Sciences
*The Role of alg10 in N-Linked Glycosylation in
Embryonic Development in Drosophila*

66) Scott Tibbetts, Biological Sciences (S&E)
Erica Selva, Biological Sciences
Defining the Wingless Binding Domain of Sprinter

67) Alicia Liu, Biological Sciences (UR)
Erica Selva, Biological Sciences
*The Effects of the O-xylosyltransferase (oxt) Mutation on Wg,
Hh, and Dpp/TGFβ Signaling*

68) John Jeffrey Mosko, Biological Sciences (INBRE)
Thomas Shaffer, Nemours Pediatric Lung Center
*Effects of Xenon Gas on Human Airway Epithelial Cells
During Post Hypoxia-Ischemia Hypothermia*

69) Danielle McKenna, Biological Sciences (INBRE)
Robert Sikes, Biological Sciences
*Which Risk Model or Combination of Risk Models is Most
Accurate in Predicting a Mutation in the BRCA1 and
BRCA 2 Genes?*

70) Brenda Mogere, Nursing (DoD) (DSU)
Robert Sikes, Biological Sciences
*Effects of ATP and Adenosine on Medistatic Prostate Cancer
Cell Line (C42-V4)*

71) Alexa Sadreameli, Biological Sciences (INBRE)
Katia Sol-Church, Nemours-Biomedical Research
Determining Parental Origin of Costello Syndrome

72) Lydia Bonar, Biological Sciences (Charles Peter White)
Jia Song, Biological Sciences
*Small RNA Regulation of Neurogenesis in the Early
Development of the Sea Urchin*

73) Megan Dumas, Biological Sciences (S&E)
Jia Song, Biological Sciences
*Small GTPase Protein Expression and their Regulation by
MicroRNA-31 during Sea Urchin Development*

74) Lauren Sager, Quantitative Biology (S&E)
Jia Song, Biological Sciences
*The mRna Localization of Potential miR-31 Gene Targets
within the Sea Urchin Embryo*

75) Adam Horn, Biological Sciences (Charles Peter White)
Kenneth van Golen, Biological Sciences
*Generation of a Stably Expressing Tetracycline Inducible
Human Mammary Epithelial Cell Line*

76) Lyana Labrada, Biological Sciences (Stetson)
Kenneth van Golen, Biological Sciences
Expression of Hyaluronidases in Prostate Cancer Cell Lines

77) Yanique Conie (DoD) (DSU)
Kenneth van Golen, Biological Sciences
*Determining the Sensitivity of IBC cells to Imatinib Using
ATP-CRA Assay*

78) Rahmeek Perry, Biology (EPSCoR) (DSU)
Cherese Winstead, DSU - Biology
*Oxytocin-loaded Chitosan Nanoparticles as a Novel Drug
Delivery System for Autism Treatment*

NEUROSCIENCE

79) Kelsey Holiday, Neuroscience (S&E)
Amy Griffin, Psychology
*Comparing the Effects of Hippocampal Inactivation between
Two Tasks that Differ on Working Memory Demand*

80) Sarah Wells, Neuroscience (S&E)
James Hoffman, Psychology
*Can the Two Cerebral Hemispheres Independently Track
Moving Objects?*

81) Kerry Criss, Neuroscience (S&E)
Anna Klintsova, Psychology
*Behavioral Therapy to Ameliorate the Effects of
Neonatal Alcohol Exposure on Dendritic Spines in the
Rodent mPFC*

82) Alejandro Morales, Neuroscience (McNair)
Anna Klintsova, Psychology
*Effects of Neonatal Alcohol Exposure on Synaptic Integration
of Newly-born Granule Cells in the Adult Dentate Gyrus in a
Rodent Model of FASD*

83) Kenneth Chen, Neuroscience (McNair)
Tania Roth, Psychology
Assessment of bdnf DNA Methylation in the Developing Amygdala Associated with Caregiving

84) Hannah Volpert, Neuroscience (S&E)
Robert Simons, Psychology
Brain Potentials Shine a Light on Implicit Racial Prejudice

85) Andrea Druga, Neuroscience (S&E)
Mark Stanton, Psychology
Context Conditioning in Weanling Rats is Dependent on NMDA Receptors in the Hippocampus

86) Alexander Gilchrist, Neuroscience (S&E)
Mark Stanton, Psychology
Short-Delay, Long-Delay, and Trace Fear Conditioning in a Neonatal Alcohol Rodent Model

NATURAL SCIENCES - (Chemistry and Biochemistry, Mathematical Sciences, Physics, Geography, Geological Sciences, Environmental Science)

CHEMISTRY AND BIOCHEMISTRY

87) Michael Ghidiu, Chemistry (Chemistry Alumni)
Joel Rosenthal, Chemistry and Biochemistry
Use of Solar Energy to Drive Chemical Fuel Production using Gold N-Heterocyclic Carbene Complexes

88) Justin Teesdale, Chemistry (Chemistry Alumni)
Joel Rosenthal, Chemistry and Biochemistry
BODIPY Appended Rhenium based Molecular Platforms for Photocatalytic CO2 Reduction

89) Steven Francisco, Chemical Engineering (CCEI Summer Fellows)
Joel Rosenthal, Chemistry and Biochemistry
The Electrocatalytic Reduction of CO2 by Ni/Co Macrocycle Complexes

90) Cory Anson, Chemistry (S&E)
Douglass Taber, Chemistry and Biochemistry
A Metal-free Route for the Synthesis of Beta Substituted Alkyl Cyclopentanones

91) Antione Jackson, Chemistry (EPSCoR) (DUS)
Qiquan Wang, DSU - Chemistry
Determination of Plastic Film Permeability to Fumigants

92) Keywan Johnson, Chemistry (HHMI/NUCLEUS)
Donald Watson, Chemistry and Biochemistry
Studies Toward Development of a Practical Silyl Heck Reaction

93) Sergio Rodriguez, Chemistry (HHMI/NUCLEUS)
Donald Watson, Chemistry
Progress Towards the Copper-Catalyzed C-Alkylation of Nitroalkanes

94) Kara Martin, Chemistry (HHMI)
Neal Zondlo, Chemistry and Biochemistry
Biological Ligations of Peptides

95) Monica Pirigyi, Biochemistry (HHMI)
Neal Zondlo, Chemistry and Biochemistry
Bi-orthogonal Ligations of Peptides and Proteins

96) Jon Tomczak, Chemistry (Chemistry Alumni)
Brian Bahnson, Chemistry and Biochemistry
Association of Plasma PAF-AH to Lipoprotein Fractions

97) Gregory Darone, Chemistry (Chemistry Alumni)
Svilen Bobev, Chemistry and Biochemistry
Synthesis and Crystal Structures of Eu14CdAs11 and Eu14CdSb11: Two Different Defect Variants of the Ca14AlSb11 StructureType

98) Shannon Owings, Chemistry (Chemistry Alumni)
George Luther, School of Marine Science and Policy
Pyrite and Elemental Sulfur Nanoparticles in the Chesapeake Bay

99) Matthew White, Biochemistry (HHMI)
Charles Riordan, Chemistry and Biochemistry
Synthesis of Nickel (I) 3-phenyl,5-methyltrispyrazolylborate and Creation of Novel Nickel Compounds by Activation of Elemental Sulfur

100) Liedeke Sweitzer, Biotechnology (INBRE) (DTCC)
Sharon Rosovsky, Chemistry and Biochemistry
Optimization of the Tobacco Etch Virus Protease's Expression and Purification

101) Elyssa Bernfeld, Biochemistry (Chemistry Alumni)
Sharon Rozovsky, Chemistry and Biochemistry
An Efficient Protocol for Sulfur Substitution by Selenium in Augmenter of Liver Regeneration

102) Soma Jobbagy, Biochemistry (Beckman)
John Koh, Chemistry and Biochemistry
Characterization of Novel Anti-androgens as Potential Prostate Cancer Therapeutics

103) Sharon (Min) Song, Chemistry/Biochemistry (McNair/INBRE)
John Koh, Chemistry and Biochemistry
Covalent Modification of the Coactivator Site of the Human Androgen Receptor

104) Brezelle Bey, Biology (DoD) (Lincoln)
John Koh, Chemistry and Biochemistry
Intein-based Expression and Labeling of the Androgen Receptor Ligand Binding Domain

105) Nicholas Audette, Biochemistry (HHMI)
Tatyana Polenova, Chemistry and Biochemistry
NMR Analysis of Actin Binding Protein Cofilin

106) Gabriel Fernandez, Biological Chemistry (INBRE) (Wesley)
Malcolm D'Souza, Wesley-Chemistry
Nucleophilic Substitution Reactions of 2-methoxyphenyl Chloroformate

107) Aaron Givens, Biological Chemistry (INBRE) (Wesley)
Malcolm D'Souza, Wesley-Chemistry
Analysis of Estrogenic Hormones in Poultry Waste

108) Brett Sansbury, Biological Chemistry (INBRE) (Wesley)
Malcolm D'Souza, Wesley-Chemistry
Understanding the Solvent Reaction of 4-Chlorophenyl Chlorothionoformate

109) John Feick, Biochemistry (S&E)
Joseph Fox, Chemistry and Biochemistry
Efficient and Versatile Bioconjugation Using Electron-Deficient Tetrazine: a Strain-driven Reaction

110) Christine Dang, Biological Sciences and Kammas Murphy, Chemistry Education (UR)
Meredith Wesolowski and Mary Beth Kramer, Chemistry and Biochemistry
Designing Interdisciplinary Intro BISC-CHEM Labs

111) Jessica Lewis, Biomedical Engineering and Audrey Guyer, Biomedical Engineering (UR)
Meredith Wesolowski and Mary Beth Kramer, Chemistry and Biochemistry
Designing Interdisciplinary Intro BISC-CHEM Labs

112) Amanda Grigoli, Chemistry (HHMI/NUCLEUS)
Zhihao Zhuang, Chemistry
Characterization of Domain Structure of a Deubiquitylating Enzyme Complex

113) Melissa Earley, Mathematics (INBRE) (Wesley)
Malcolm D'Souza, Wesley-Chemistry
Enhancing the Public's Knowledge of Cancer Drugs and their Characteristics

MATHEMATICAL SCIENCES

114) Liu Chang, Mathematics (EPSCoR) (DSU)
Liang Liu, DSU - Mathematics
Reconstructing the Posterior Distributions of a Species Phylogeny Estimating the Evolutionary History of Humans

115) Dajun Lin, Mathematics (S&E)
Sebastian Cioaba, Mathematical Sciences
An Attempt to Find Cospectral Mates to K(9,3)

116) Jennifer Bruhns, Quantitative Biology (HHMI) and Douglas Freeman, Mathematics Education
Richard Braun, Mathematical Sciences
Models for Osmosis Between the Cornea and the Tear Film

117) Colleen Moens, Quantitative Biology (HHMI)
Pak-Wing Fok, Mathematical Sciences
The Effect of LDL Dynamics on Cell Counts in Atherosclerotic Plaque

PHYSICS

118) Eric Sabo, Mathematics (S&E)
Qaisar Shafi, Physics and Astronomy
Inflation in the Era of Precision Cosmology

119) Willow Crosby, Physics (S&E)
Barry Walker, Physics and Astronomy
Relativistic Photoelectrons from Neon

120) Jason Huynh, Physics (S&E)
George Hadjipanayis, Physics and Astronomy
Iron-Platinum Nanoparticles for Biomedical Applications

121) Rohan Patel, Physics (HHMI/NUCLEUS)
Edward Lyman, Physics and Astronomy
Structure and Dynamics of the A2a Adenosine G-Protein Coupled Receptor

GEOGRAPHY

122) Jessica McKnight, English/Communication (S&E)
David Legates, Geography
Dissemination of Environmental Information to the General Public

123) Sharon Nebbia, Environmental Science (EPSCoR)
David Legates, Geography
Tidal Water Level Monitoring on the Delaware Coast

124) Shannon McElhinney, Environmental Science (EPSCoR)
Lodevicus Claessens, Geography
Historical Waterbudget of the White Clay Creek Watershed

GEOLOGICAL SCIENCES

125) Deon Knights, Geology (UUS/EPSCoR)
Holly Michael, Geological Sciences
Analysis of Pure Water Chemistry Along Indian River, Delaware

126) Andrew Musetto, Environmental Science (EPSCoR)
Holly Michael, Geology
Testing CMT Well Effectiveness and Characterizing Porewater Salinity Profiles

ENVIRONMENTAL SCIENCE

127) Kathleen Harris, Environmental Science (S&E)
Jeremy Firestone, School of Marine Science and Policy
Public Opinions on the Process of Offshore Wind Power Implementation and Its Effect on the Support for Offshore Wind Power

128) Taylor Hendricks, Environmental Science (EPSCoR) (Wesley)
Bruce Allison, Wesley - Environmental Science
A Water Quality Assessment of Nontidal Streams in the Sassafras River Watershed

129) Greg McKee, Environmental Science (EPSCoR) (Wesley)
Bruce Allison, Wesley - Environmental Science
Using Watershed and Field Level GIS and Computer Modeling Applications to Evaluate Watershed Health

130) Eddie Meade, Environmental Science (EPSCoR) (Wesley)
Bruce Allison, Wesley - Environmental Science
A Sub-watershed Analysis of Factors Influencing the Potential for Stream Channel Restoration at the Stockley Center

131) Melissa Savin, Environmental Science (EPSCoR) (Wesley)
Bruce Allison, Wesley - Environmental Science
Experiential Learning through Environmental Outreach: An Undergraduate Student Perspective

ENGINEERING – (Bioresources Engineering, Chemical Engineering, Civil and Environmental Engineering, Electrical and Computer Engineering, Computer and Information Sciences, Mechanical Engineering, Materials Sciences)

BIORESOURCES ENGINEERING

132) Bianca Morales, Environmental Engineering (CANR)
Carmine Balascio and Anastasia Churnside, Bioresources Engineering
Automated Water Quality Sampling for Evaluation of an Advanced Bioretention System

CHEMICAL ENGINEERING

133) Erik Anderson, Chemical Engineering (CCEI Summer Fellows)
Douglas Buttrey, Chemical Engineering
Oxidative Dehydrogenation of Butene to Butadiene Employing Multi-Component Molybdates

134) Yifei Liu, Chemical Engineering (Northeastern Chemical Association)
Douglas Buttrey, Chemical Engineering
Continuous Separation of Hydroxymethylfurfural (HMF) from Aqueous Solution by Y-shaped Microseparator

135) Adam Bivens, Chemical Engineering (CCEI Summer Fellows)
Jingguang Chen, Chemical Engineering
Synthesis of Nickel Borides as Potential Catalysts for the Electrochemical Hydrogen Evolution Reaction (HER) in Alkaline Solutions

136) Scott Ehrmann, Chemical Engineering (S&E)
Jingguang Chen, Chemical Engineering
Reactor Studies on Bimetallic Catalysts

137) Lisa Thomanek, Chemical Engineering (CCEI Summer Fellows)
Jingguang Chen, Chemical Engineering
DFT Calculations of Nitrogen Binding Energy on Bimetallic Surfaces

138) Zachary March, Chemical Engineering (HHMI)
David Colby, Chemical Engineering
Development of an Amyloid Seeding Assay for Sensitive Detection of Misfolded Tau

139) Brittany Earnest, Biochemical Engineering (NISE REU) (UMD)
David Colby, Chemical Engineering
Methods of Culturing Primary Mouse Neurons Towards the Analysis of a Cellular Huntington's Disease Model

140) Ryan O'Boyle, Biomedical Engineering (S&E)
David Colby, Chemical Engineering
Epitope Mapping the Prion Protein

141) Siming Li, Chemical Engineering (CCEI Summer Fellows)
Weihua Deng, Catalysis Center for Energy Innovation
Design and Building of a Fixed Bed Reactor System

142) Ronald Lewis, Chemical Engineering (S&E)
Thomas Epps, Chemical Engineering
Block Copolymer Thin Film Processing

143) Joey Kim, Chemical Engineering (UR)
Thomas Epps, Chemical Engineering
Characterization of a Monolayer Gradient Vapor Deposition Device

144) Sarah Hann, Chemical Engineering
Thomas Epps and Millicent Sullivan, Chemical Engineering
Stabilization and Functionalization of Self-Assembled Micelles

145) Robert Pagels, Chemical Engineering (S&E)
Millicent Sullivan, Chemical Engineering
pH-Responsive Amphiphilic Polymer-Peptide Conjugates for Drug Delivery

146) Touseef Habib, Chemical Engineering (CCEI Summer Fellows)
Feng Jiao, Chemical Engineering
Photo-catalysts: Manganese in Various Silica Supports

147) Hassan Siddiqui, Chemical and Biomolecular Engineering (NISE REU) (UPENN)
Eric Furst, Chemical Engineering
Electrokinetic Properties of Polyelectrolyte Coated Colloidal Particles

148) Kameron Conforti, Chemical Engineering (Northeastern Chemical Association)
Feng Jiao, Chemical Engineering
Synthesis and Characterization of Li2CoSiO4/C as a Cathode Material for Li-ion Batteries

149) Brian Leone, Chemical and Biomolecular Engineering (CCEI Summer Fellows) (UPENN)
Jingguang Chen, Chemical Engineering
Catalytic Activation of Carbon Dioxide

150) Amy Quach, Chemical Engineering (CCEI Summer Fellows)
Michael Klein, Chemical Engineering
Discrimination between Free-Radical and Concerted Mechanisms in Hydrocarbon Pyrolysis

151) Eric Macedo, Chemical Engineering (UR)
April Kloxin, Chemical Engineering
Hydrogels for Controlling the Cell Microenvironment: Fibrous Materials for Understanding and Directing Ligament Regeneration

152) Catherine Halat, Chemical Engineering (CCEI Summer Fellows)
Vladimiros Nikolakis, Catalysis Center for Energy Innovation
Glucose, Fructose, and Mannose Solubility in Methanol and 2-Butanol

153) Andrew Shah, Chemical Engineering (CCEI Summer Fellows)
Vladimiros Nikolakis, Catalysis Center for Energy Innovation
Investigation of the Interactions between Glucose and Fructose with Dimethylsulfoxide (DMSO) – Water – CrCl3 Mixtures using Vibrational Spectroscopy

154) Peter Terpeluk, Chemical and Biomolecular Engineering (CCEI Summer Fellows) (UPENN)
Vladimiros Nikolakis, Catalysis Center for Energy Innovation
Investigation of Liquid Phase 1,2-Propanediol Dehydration

155) Andrew Bitner, Chemical Engineering (S&E)
Babatunde Ogunnaike, Chemical Engineering
Stochastic Modeling of Glycosylation Concentration Distribution in Monoclonal Antibodies

156) Meng Ren, Chemical Engineering (faculty funded)
Eleftherios Papoutsakis, Chemical Engineering
Heterologous Expression of Wood-Ljundahl Pathway Genes into C. acetobutylicum

157) Sintia Krizman, Biochemistry (HHMI)
Christopher Roberts, Chemical Engineering
Biophysical Nature of Non-ionic Surfactants as Protein Stabilizers

158) Nicolas Di Domizio, Chemical Engineering (S&E)
Anne Robinson, Chemical Engineering
Insertion of a Monomeric Cyan Fluorescent Protein into Yeast Wild-Type and Human Chimeras of the G Protein α Subunit for Visualization in S. cerevisiae

159) Michael Orella, Chemical Engineering (CCEI Summer Fellows/S&E)
Dionisios Vlachos, Chemical Engineering
Fructose Dehydration into 5-hydroxymethylfurfural (HMF): Kinetics and Thermodynamics

160) Andrew Speese, Chemical Engineering (CCEI Summer Fellows)
Dionisios Vlachos, Chemical Engineering
Insights into the Thermal Decomposition of Ethanol on Pt(111) via Density Functional Theory Calculations

161) Anthony Pallanta, Chemical Engineering (UR)
Norman Wagner, Chemical Engineering
The Use of Shear Thickening Fluids in Impact-Resistant Materials

CIVIL AND ENVIRONMENTAL ENGINEERING

162) Brittany DeBord, Environmental Engineering (EPSCoR)
Julia Maresca, Civil and Environmental Engineering
Effects of the Quorum-quenching Gene of Sulfolobus solfataricus on Mineral Degradation

163) Julianne Page, Environmental Engineering (EPSCoR)
Julia Maresca, Civil and Environmental Engineering
Behaviors and Classification of Coastal Bacteria

164) Jordan Wynn, Civil Engineering (S&E)
Jennifer McConnell and Harry Shenton, Civil and Environmental Engineering
Evaluation of Unpainted Weathering-Steel Highway-Bridge Performance

165) Lindsey Olson, Environmental Engineering (NISE REU)
Daniel Cha, Civil and Environmental Engineering
Engineered Rapid Infiltration System for Enhanced Removal of Nitrate from Middletown Wastewater Treatment Plant Secondary Effluent

166) James Comiskey, Environmental Engineering (EPSCoR)
Steven Dentel, Civil and Environmental Engineering
Methane Attenuation Strategies for Greenhouse Gas Sequestration by Geothermal Biodegradation

167) Anthony McGuire, Environmental Engineering (EPSCoR)
Pei Chiu, Civil and Environmental Engineering
Replacing Chlorine with Nanoscale Zero-Valent Iron-Coated Media for Point-of-Use Drinking Water Treatment

168) Brandon Witt, Environmental Engineering (NISE REU)
Paul Imhoff, Civil and Environmental Engineering
Effects of Poultry Litter Biochar on Soil-Water Properties in an Agricultural Soil

ELECTRICAL AND COMPUTER ENGINEERING

169) Thomas Potter, Electrical Engineering (S&E)
Sylvain Cloutier, Electrical and Computer Engineering
Analysis of the Efficiency and Life Span of Polymer Based Solar Cells

170) Nicole Wells, Electrical Engineering (S&E)
Fouad Kiamilev, Electrical and Computer Engineering
Vehicle-Grid Communication: Interfacing Electric Vehicles with the Smart Grid

171) Ryan Lee, Electrical and Computer Engineering (Rowan/UD REU) (Rowan)
Dennis Prather, Electrical and Computer Engineering
Fabrication of Dual RF-Optical Horizontal Slot Waveguides

172) David Koeplinger, Electrical Engineering (S&E)
Takashi Buma, Electrical and Computer Engineering
Photoacoustic Microscopy with a Multicolor Stimulated Raman Scattering Pulsed Optical Source

173) David Calhoun, Electrical and Computer Engineering (Rowan/UD REU) (Rowan)
Mark Mirtoznik, Electrical and Computer Engineering
Design of Wideband Structural Radomes

174) Yang Yu, Electrical Engineering (S&E)
Joshua Hertz, Mechanical Engineering
Preparation and Characterisation of Lanthanum Silicates by a Sol-gel Process

175) Akshat Goyal, Mechanical Engineering (NISE REU) (Northeastern)
Kenneth Barner, Electrical and Computer Engineering
Mechanical Properties of Organs for Virtual Surgery Simulation

COMPUTER SCIENCE

176) Michelle Allen, Computer Science (NSF REU)
Lori Pollock and Vijay Shanker, Computer and Information Sciences
Automatically Splitting Identifiers in Java Programs

177) Cory Bart, Computer Science (Summer Scholars/S&E)
Lori Pollock and Vijay Shanker, Computer and Information Sciences
Reversing Engineering from Java Identifier Names: Conventions and a Grammar

178) Jeff Wise, Computer Science (NSF REU) (Franklin and Marshall)
Lori Pollock and Vijay Shanker, Computer and Information Sciences
Detecting High-Level Actions in Java Code for Automatic Comment Generation

179) Matthew Saponaro, Computer Science (S&E)
Keith Decker, Computer and Information Sciences
Dynamics of Colon Cancer Development: an Agent-based Computational Model

180) Matthew Stagitis, Computer Science (S&E)
Sandra Carberry, Computer and Information Sciences
Exploiting Information Graphics in a Digital Library: Constructing a Development Set

181) Matthew Wezowicz, Computer Science (S&E)
Michela Taufer and David Saunders, Computer and Information Sciences
Multicore Acceleration of Matrix Multiplication in Linbox Using OpenCL

182) Michael Matheny, Computer Science (UR)
Michela Taufer, Computer and Information Sciences
Using Games in Volunteer Computing to Promote Participation in the Exscitech Project

183) Samuel Schlachter, Computer Engineering (UR)
Michela Taufer, Computer and Information Sciences
Developing a Powerful Simulation Interface for Efficiently Placing Low-Cost QCN Sensors

MECHANICAL ENGINEERING

184) Akiva Landsman, Physics (NISE REU) (Brandeis)
Sunil Agrawal, Mechanical Engineering
Design of an Intuitive Interface for Driving a Pediatric Robotic Walker Steered with Trunk Motion

185) Neelima Agrawal, Communication Sciences and Disorders (NISE REU) (Northwestern)
Sunil Agrawal, Mechanical Engineering
Adaptation and Retention in Gait Function of Healthy Adolescents with Use of the Tethered Pelvic Assistive Device

186) Stephen Hale, Mechanical Engineering (S&E)
Suresh Advani, Mechanical Engineering
Thermal Conductivity Enhancement of LaNi5 Metal Hydride

187) Molly Wessel, Biomedical Engineering (S&E)
Jill Higginson, Mechanical Engineering
Effects of Handrail Forces in Treadmill Walking of Post-Stroke Patients

188) Benjamin Hockman, Mechanical Engineering (S&E)
Herbert Tanner, Mechanical Engineering
Emulating Radioactive Emissions with a Pulsed Laser

189) Nicholaus Lacock, Computer Science (S&E)
Herbert Tanner, Mechanical Engineering
Implementation of Model Predictive Control for Robot Navigation

190) Christine Gregg, Mechanical Engineering (S&E)
Erik Thostenson, Mechanical Engineering
Characterization of Damage Mechanisms in Mechanically Fastened Composite Joints Using Carbon Nanotube Networks

191) Matthew Sinnott, Mechanical Engineering (S&E)
Erik Thostenson, Mechanical Engineering
Vascular Self-Healing and Damage Detection in Braided Composites

MATERIALS SCIENCE

192) Peter Attia, Chemical Engineering (S&E)
Joshua Zide, Materials Science and Engineering
Thermoelectric Power Generation in Time-varying Temperature Environments

193) Thomas Cristiani, Chemical Engineering (S&E)
Joshua Zide, Materials Science and Engineering
Thermodynamic Modeling of Concentration Gradients in InGaAs Quantum Dots

194) Ryan DelPercio, Environmental Science (EPSCoR) (DTCC)
Ismat Shah, Materials Science and Engineering
Synthesis and Characterization of Self-Cleaning Nano-Porous Oxide Filters for Water Purification

195) Katy Mazer, Biological Engineering (NISE REU) (North Carolina State)
Ismat Shah, Materials Science and Engineering
The Use of Titanium Dioxide in Organic Solar Cells

196) Mark McGarity, Mechanical Engineering (NISE REU) (Villanova)
Ismat Shah, Materials Science and Engineering
High Surface Area Nanoporous Ti02 Coating for Effective Water Condensation

CENTER FOR COMPOSITE MATERIALS

197) Devin Prate, Mechanical Engineering (CCM)
Johnhwan Suhr, Mechanical Engineering
Investigation of the Aspect ratio Effects of Glass Fiber in Polycarbonate Composites

198) Britannia Vondrasek, Ocean Engineering (CCM)
(Univerity of Vermont)
Erik Thostenson, Mechanical Engineering
Processing of Nanostructural Electroactive Composite Films for Sensing Applications

199) Andrew Beauchemin, Mechanical Engineering (CCM)
Bazle Haque, Center for Composite Materials
Modeling the Ballistic Penetration of Thick-Section Composites on a Rigid Support

200) Kayla Dunbar, Mechanical Engineering (CCM) (Southern University and A&M College)
Bazle Haque, Center for Composite Materials
Experiment and Modeling of Sandwich Composites Under Impact Loading Conditions

201) Richard Readdy, Mechanical Engineering (CCM)
Suresh Advani, Mechanical Engineering
Automation of the VARTM Process using Electromagnetism

HUMAN SCIENCES – (Medical Technology, Physical Therapy, Kinesiology and Applied Physiology)

MEDICAL TECHNOLOGY

202) Michelle Francis, Medical Technology (INBRE)
Mary Ann McLane, Medical Technology
Crosslinking Venom Protein to Melanoma Cells

203) Brendan Mooney, Medical Technology (S&E)
Mary Ann McLane, Medical Technology
Phosphotyrosine Immunoprecipitation in WM164 Melanoma Cells after Eristostatin Treatment

204) Dwaipayan Muhuri, Medical Technology (S&E)
Mary Ann McLane, Medical Technology
Intracellular Phosphorylation in Melanoma Cells Exposed to Disintegrin Eristostatin

205) Ryan Penn, Medical Technology (INBRE)
Mary Ann McLane, Medical Technology
Examining the Effects and Interactions of Eristostatin and Fibronectin on Melanoma Cells in Three-Dimensional Environments

PHYSICAL THERAPY

206) Shreya Jammula, Biological Sciences/Neuroscience
(Life Sciences)
Stuart Binder-Macleod and Darcy Reisman, Physical Therapy
Role of BDNF on Neuronal Plasticity Post Stroke

207) Alexandra Lambert, Biological Sciences
(Charles Peter White)
Katherine Rudolph, Physical Therapy
Movement Strategies Associated with Instability during Lateral Stepping in Knee Osteoarthritis

208) Katharine McWilliams, Exercise Science (Charles Peter White)
Katherine Rudolph, Physical Therapy
Joint Kinetics and Kinematics during a Step-Down in People with Knee Osteoarthritis

209) Dana McCoy, Exercise Science (Life Sciences)
Darcy Reisman, Physical Therapy
Effects of Activity Monitoring on Community Ambulation and Clinical Measures of Activity in People Post-Stroke

210) Alexia Hay, Exercise Science (INBRE)
Lynn Snyder-Mackler, Physical Therapy
Effect of Anterior Cruciate Ligament (ACL) Injury on Vertical Ground Reaction Forces during Walking in Non-copers

211) Victoria Allen, Nursing (UR)
Lynn Snyder-Mackler, Physical Therapy
Participation in Level I/II Activities by Potential Copers and Non-copers Twelve Months after Anterior Cruciate Ligament Reconstruction

212) Michele Wallace, Exercise Science (McNair)
Lynn Snyder-Mackler, Physical Therapy
Performance in Functional and Unstable Non-Copers with ACL Deficiency

KINESIOLOGY AND APPLIED PHYSIOLOGY

213) Shirleeah Fayson, Athletic Training (McNair)
Thomas Kaminski, Kinesiology and Applied Physiology
The Effect of Kinesio Tape® on Muscle Activation During a Jump Landing Maneuver

214) Maggie Griebert, Athletic Training (S&E)
Thomas Kaminski, Kinesiology and Applied Physiology
The Effect of Kinesio® Tape on Plantar Pressure in People with Medial Tibial Stress Syndrome

215) Sara Naguib, Exercise Science (INBRE)
Thomas Kaminski, Kinesiology and Applied Physiology
Examination of Foot Parameters in Subjects with Chronic Ankle Instability and in Subjects with Stable Ankles

216) Zachary Mease, Exercise Science (INBRE)
David Edwards, Kinesiology and Applied Physiology
The Effect of Arginase Inhibition and L-Arginine Treatment in Rats with Early or Late Stage Chronic Kidney Disease

217) Colin Stanhope, Exercise Science (INBRE)
David Edwards, Kinesiology and Applied Physiology
Cardiovascular Response to Passive Whole Body Temperature Stress

218) Brian Crossan, Exercise Science and Traci LaBerge,
Exercise Science (INBRE) (DTCC)
Mark Lafferty, DTCC-Exercise Science
Customized Cardiovascular Exercise Prescription: Effects of a 30 Minute Interval Training Design Based on Pretest Anaerobic Threshold and VO2max Values

219) Audrey Companiony Hopkins, Exercise Science (INBRE)
James Richards, Kinesiology and Applied Physiology
Validation of Palpation as a Method to Identify Scapular Bony Landmarks

220) Kevin Rowley, Exercise Science (S&E)
James Richards, Kinesiology and Applied Physiology
Identification of Scapular Kinematics using Surface Mapping

221) Josh Wallace, Exercise Science (S&E)
Bill Rose, Kinesiology and Applied Physiology
Forearm Versus Upper Arm Blood Pressure Differences

222) Erica Hartman, Biochemistry (S&E)
Christopher Modlesky, Kinesiology and
Applied Physiology
The Effects of High-frequency Low-magnitude Vibration on Muscle Activity in Children with Osteogenesis Imperfecta

SOCIAL SCIENCES, HUMANITIES, & ARTS – (Psychology, Business Administration, ArtsBridge, Education)

PSYCHOLOGY

223) Elizabeth Allen, Psychology (S&E)
Mary Dozier, Psychology
Promoting Target Behaviors: The Role of Therapist Feedback

224) Kaitlin Flannery, Psychology (S&E)
Mary Dozier, Psychology
Effect of Neglecting Mothers' Attachment State of Mind on Children's Competence

225) Quindara Lazenbury, Psychology Education (UUS)
Carroll Izard, Psychology
Self-efficacy and Culturally Relevant Positive Statements

BUSINESS ADMINISTRATION

226) Karoline Guerrero, Marketing (McNair)
Daniel Freeman, Business Administration
Co-shopping with Luxury Brands

227) Dana Griskowitz, Sports Management (SL Fellow)
Matthew Robinson, Business Administration
Delaware Olympic Day 2011

ARTSBRIDGE

228) Sarah Janus, History and Jennifer Ryan, Elementary
Teacher Education (ArtsBridge)
Lynnette Overby, UREL/Theatre
Community Partner: Bunker Hill Elementary School,
Appoquinimink School District
*Assessing the Living History Curriculum: Connecting
Colonial History with Dance and Drama*

229) Julie Luzier, Cognitive Science (ArtsBridge)
Lynnette Overby, UREL/Theatre
Community Partner: Early Learning Center
*The Effects of Movement on the Speech and Communication
Skills of Preschool Students*

230) Pamela Oppenheimer (ArtsBridge)
Lynnette Overby, UREL/Theatre
Community Partner: Cab Calloway Summer School for
the Arts
*Assessing Arts and Humanities Content: Cab Calloway
Summer School for the Arts (Weeks 1, 3 and 5)*

231) Matthew Jufer, Music Education (ArtsBridge)
Lynnette Overby, UREL/Theatre
Community Partner: Cab Calloway Summer School for
the Arts
*Assessing Arts and Humanities Content: Cab Calloway
Summer School for the Arts (Weeks 2, 4 and 7)*

EDUCATION

232) Rachelle Bull, Math Education and Kelly Buckley,
Psychology Education (SL Fellows)
Melva Ware, Delaware Center for Teacher Education
Clearing the Pathway to College

233) Elise Jackson, English Education (SL Fellow)
Melva Ware, Delaware Center for Teacher Education
Reading and Motivation: What Students Want and Need

234) Leo DeRita, Chemical Engineering (UR)
Norman Wagner, Chemical Engineering
Pluronic Solutions and Self Assembly in Ionic Liquids

235) Joseph Angeloni, Chemical Engineering (UR)
(Clemson University)
Norman Wagner, Chemical Engineering
Lysozyme Cloud Point Analysis

9:00 – 9:50   *Oral Session 1*

CHILDREN AND THE ARTS   (ROOM 121)

Moderator: Philip Gentry, Music

Rebecca Cweibel, Music Education (Arts)
Philip Gentry, Music
*"I Just Love Good Music": Leonard Bernstein's Young People's
Concerts and the Middlebrow*

Gregory Casement, Jenna Knaster, and Mathew Marion,
Music Education (Service-Learning)
Suzanne Burton, Music
Community Partners: Red Clay School District (Richardson Park Elementary), and Christina School District
(Shue-Medill Middle School and Thurgood Marshall
Elementary)
*ProjectMUSIC: Teaching-Artists Engaging Students
and the Community*

Kristen Dempsey, English (Humanities)
Allan Carlsen, Theatre
The Evolution and Importance of Children's Theatre

HEALTH AND POLICY   (ROOM 122)

**Moderator: Erlinda Wheeler, Assistant Director,
School of Nursing**

Gealina Dun, Neuroscience (Social Sciences)
Marian Palley, Political Science & International Relations
*Understanding and Navigating Health Insurance for
Families of Children with Cerebral Palsy*

Brittany Drazich, Nursing (Service-Learning)
Bethany Hall-Long, Nursing
Community Partners: Christiana Care Health System and
Nemours/Alfred I. duPont Hospital for Children
*National Children's Study: Pregnant Teens and
Non-resident Fathers*

Gabrielle Simonette, Early Childhood Education
(Social Sciences)
Steve Eidelman, Human Development and Family Studies
*Defining Progressive Residential Services for Adults with
Disabilities: A Leadership Perspective*

LITERATURE (ROOM 123)

Moderator: D. Heyward Brock, English

Hans Howk, English (Humanities)
Siobhan Carroll, English
Beyond the Realm of Land: The Hot Air Balloon and its Effect on Western Imagination in Literature

Samantha Toscano, English (Humanities)
Heyward Brock, English
A Revised Ben Jonson Companion

Gregory Laluna, English (Humanities)
Christopher Penna, English
"Nature's Logic": Understanding Tragedy in the Works of Thomas Hardy

EXERCISE AND HEALTH (ROOM 124)

**Moderator: Nancy Getchell,
Kinesiology and Applied Physiology**

Talia Baldini, Health Behavior Science and Jocelyn Dressel, Health Studies (Service-Learning)
Iva Obrusnikova, Behavioral Health and Nutrition
Community Partners: Delaware Adapted Sports Club and Paws for People
The Effects of Therapy Dog-assisted Exercise on Physical Activity of Children with Autism Spectrum Disorders

Kayla Carrigan, Psychology, Amanda Gropack, Exercise Physiology, and Jennifer Murphy, Exercise Science (Service-Learning)
Nancy Getchell, Kinesiology and Applied Physiology
Community Partner: Delaware Breast Cancer Coalition
Developing an Exercise Program for Women with Breast Cancer in Delaware

Angelica Montes, Medical Technology (McNair)
Barret Michalec, Sociology and Criminal Justice
The Role of Medical Interpreters in Health Care Delivery

10:00 – 10:50   *Oral Session 2*

MUSIC AND HUMAN BEHAVIOR (ROOM 121)

Moderator: Suzanne Burton, Music

Ignacio Angulo-Pizarro, Music Education (McNair)
Duane Cottrell, Music
An Examination of Ensemble Rehearsal Technique

Jacqueline Adams, Music Education (Arts)
Suzanne Burton, Music
Common Threads between Music and Language Development: From Process to Product

Audrey Rossi, Human Services (Social Sciences)
Nancy Weiss and Steve Eidelman, Human Development and Family Studies
Understanding the Leadership Outcomes in Participants of the Leadership Institute

INTERNATIONAL RELATIONS (ROOM 122)

Moderator: Julio Carrión, Political Science & International Relations and Director, Latin American Studies

Colleen Trowbridge, International Relations (Social Sciences)
William Meyer, Political Science & International Relations
Mexican Corporatism: The Story of a Century

Brenna James, Political Science (McNair)
Mark Miller, Political Science & International Relations
Obama's Role in the Arab-Israeli Conflict

Kelly Ehrenreich, Political Science (Social Sciences)
Matthew Weinert, Political Science & International Relations
Human Dignity and Women's Dignity in International Law

TEACHER EDUCATION (ROOM 123)

Moderator: Robert Hampel, Interim Director, School of Education

Sharline Derosier, Elementary Teacher Education (McNair)
Elizabeth Soslau and William Lewis, School of Education
Student Teachers Speak Out: Exploring Relationships between Challenges and Efficacy when Learning to Teach

Anh Nguyen, Cognitive Science/Psychology (Service-Learning)
Carol Wong, School of Education
Community Partners: Christiana High School, Glasgow High School and Newark High School
Empowering the AVID (Advancement Via Individual Determination) Community: One Voice Can Make A Change

Teresita Mariano, English Education (McNair)
Elizabeth Higginbotham, Sociology and Criminal Justice
Summer Reading Lists: How do Hispanic High School Students Respond to these Books?

HISTORICAL AND PERSONAL ECONOMICS (ROOM 124)

Moderator: Saul Hoffman, Chair, Economics

Monica Dawson, Economics (Social Sciences)
Farley Grubb, Economics
The Effectiveness of Trade Embargoes during the American Revolution

Patrick McBrearty, Economics (Social Sciences)
James Butkiewicz, Economics
A Quantitative Analysis of the War Finance Corporation

Matthew Michael, Criminal Justice (Social Sciences)
Tammy Anderson, Sociology and Criminal Justice
An Analysis of the Social Costs of Internet Gambling

11:00 – 12:00   *Oral Session 3*

ART/DANCE/JOURNALISM   (ROOM 121)

Moderator: John Cox, Art

Kathleen Skirvin, Fine Art (Arts)
Robert Straight, Art
Udel Faces

Samantha Mancuso, Fine Arts (Arts)
Jon Cox, Art
Gum Bichromate Printing & the Hadza: An Exploration of the Alternative

Sarah Janus, History Education (Humanities)
Kimberly Schroeder, Dance
Crosswalk Choreography: A Study of Pedestrian Movement and Sound through Dance

Shane Palkovitz, English (Humanities)
McKay Jenkins, English
Peasants in Paradise

MUSIC AND ART THERAPY/ ART CONSERVATION   (ROOM 122)

Moderator: Vicki Cassman, Art Conservation

Andrew Reitter, Music/Psychology (Humanities)
Philip Gentry, Music
Concertos to Concept Albums: A Music Therapy Perspective

Lindsay Schmittle, Visual Communications (Service-Learning)
Jon Cox, Art
Community Partner: Exceptional Care for Children
Marketing, Managing Art Therapy, and Mural Painting at Exceptional Care for Children

Elizabeth Knight, Material Culture Preservation (Arts)
Vicki Cassman, Art Conservation
A Common Thread: Authenticity Issues in Textile Conservation

Katie Bonanno, Material Culture Preservation (Arts)
Vicki Cassman, Art Conservation
Cutting Threads: Preserving Authenticity in Textile Conservation

POLITICS/PUBLIC POLICY   (ROOM 123)

Moderator: Danilo Yanich, School of Public Policy

Amanda Schechter, Political Science (Social Sciences)
Lindsay Hoffman, Communication
From Newspapers to Smartphones: Does New Technology Affect Political Efficacy?

Donald Roberts, Political Science (Social Sciences)
Lindsay Hoffman, Communication
The Role of Emotions in Online Political Engagement

William Humphrey, Political Science (Social Sciences)
Tracey Holden, Communication
"Dream On": American Dream Rhetoric in Ten Presidential Nomination Acceptance Speeches, 1964-2004

James Campbell, Sociology/Criminal Justice (McNair)
Gerald Turkel, Sociology and Criminal Justice
Race and Politics: The 2008 Obama Campaign and Presidency

Shantel Campbell, Communication (McNair)
Danilo Yanich, Urban Affairs and Public Policy
Déjà Vu: A Case Study of Shared Services Agreements and Their Impact on Local TV News Content

MOVEMENT AND COGNITION   (ROOM 124)

Moderator: Ann Ardis, Deputy Dean, College of Arts and Sciences

Kyle Berger and Vincent Fronczkowski, Health and Physical Education (Service-Learning)
Kristin Scrabis-Fletcher, Behavioral Health and Nutrition
Community Partner: Girls, Inc.
Integrating Academics into Physical Activity

Jennifer Ferris, Music/History Education (ArtsBridge)
Lynnette Overby, UREL/ Theatre
Community Partner: Early Learning Center
Integrating Social Studies into the First Grade Reading in Motion Curriculum

Paige Glassman, Civil Engineering, Teagan Thomas, Elementary Teacher Education, and Zakiyah Johnson, Political Science (ArtsBridge)
Lynnette Overby, UREL/ Theatre
Community Partner: Bayard Middle School
Dance and Transportation: Interdisciplinary Curriculum for Middle School Students

Amber Beaman, Elementary Teacher Education (McNair)
Lynnette Overby, UREL/Theatre
Community partner: Kuumba Academy Charter School
Two Models for Teaching Fractions

1:30—2:30 (Auditorium, Room 128)
HHMI Keynote Speaker
"Exploration and Exploitation of Mycobacteriophages"
Graham Hatfull, Eberly Family Professor and HHMI Professor and Chair of Biological Sciences at the University of Pittsburgh

1;30 – 2:30 *Oral Session 4*

HISTORY (ROOM 121)

Moderator: Lawrence Duggan, History

Julie James, History (Humanities)
Lawrence Duggan, History
Icons, Images, and Visions in Medieval and Renaissance Europe

Shannon Knee, History (Humanities)
Daniel Callahan, History
A Modern Reevaluation of the Stirrup Thesis

Jacob Samuels, History (Humanities)
Daniel Callahan, History
A Medieval Example of How Religious Toleration and Economic Liberalization Leads to Wealth

Andrew Berni, History (Humanities)
Jesus Cruz, History
The American Catholic Church and Spanish Anticlericalism: 1931-1939

CRIME AND REHABILITATION (ROOM 122)

Moderator: Ronet Bachman, Chair, Sociology and Criminal Justice

Helen Turkel, Criminal Justice (Social Sciences)
Susan Miller, Sociology and Criminal Justice
Juvenile Victimization and Offending: The Link between Youth Offending and their Victimization

Katrina Gearhart, Women's Studies/Sociology (Service-Learning)
Kathleen Turkel, Women's Studies
Community Partner: Delaware Coalition Against Domestic Violence
Domestic Violence Services and Prevention: Past, Present, and Future

Kristee Copley, Criminal Justice (Social Sciences)
Ronet Bachman, Sociology and Criminal Justice
The Effects of Motherhood on Desistance for a Drug-Involved Sample of Female Offenders

Jessica Hickman, Criminal Justice (Social Sciences) and Nikki Kress, Sociology (UR)
Crysanthi Leon, Sociology and Criminal Justice
Prostitution Diversion Project

CONSUMER BEHAVIOR AND ENVIRONMENTAL STUDIES (ROOM 123)

Moderator: Hye-Shin Kim, Director of Undergraduate Programs, Fashion and Apparel Studies

Nicole Vassallo, Apparel Design (Social Sciences)
Sharron Lennon, Fashion and Apparel Studies
Black Friday: Consumer Misbehavior from the Eye of the Retailer

Michelle Ma, Fashion Merchandising (Social Sciences)
Hye-Shin Kim, Fashion and Apparel Studies
Self-Brand Congruity and Environmental Involvement on Perceptions of Green Marketing Claims for Apparel Brands

Christine Howard, Dean's Scholar in Environmental Textiles (Social Sciences)
Huantian Cao, Fashion and Apparel Studies
Environmentally Friendly Leather Tanning

EXERCISE AND THE ELDERLY (ROOM 124)

Moderator: Susan Hall, Deputy Dean, College of Health Sciences

Kendall Poole, Exercise Science (McNair)
Christopher Knight, Kinesiology and Applied Physiology
Physical and Cognitive Quickness Training for the Elderly and Special Populations

Julie Davis and Theresa Mitchell, Health Behavior Science (Service-Learning)
Elizabeth Orsega-Smith, Behavioral Health and Nutrition
Community Partner: Claymore Center
Older Adults and Wii Fit, How Can They Balance?

Emily Berrue and Kayleigh Nydick, Health Behavior Science (Service-Learning)
Elizabeth Orsega-Smith, Behavioral Health and Nutrition
Community Partner: Howard Weston Senior Center
Can Seniors Connect with Kinect?

2:40 – 3:40 *Oral Session 5*

SCIENCE AND ENGINEERING   (ROOM 125)

Moderator: Deni Galileo, Biological Sciences

Joanna Adadevoh, Chemical Engineering (UUS)
Babatunde Ogunnaike, Chemical Engineering
The Effect of Ammonia on the Glycosylation Pattern of Monoclonal Antibodies

Soma Jobbagy, Biochemistry (Beckman)
John Koh, Chemistry and Biochemistry
Characterization of Novel Anti-androgens as Potential Prostate Cancer Therapeutics

Hamza Bhatti, Biological Sciences (HHMI)
Deni Galileo, Biological Sciences
Time Lapse Analysis of Glioma Cell Behavior in Embryonic Brain Cell Co-Cultures

Michael Brister, Biochemistry (HHMI)
Neal Zondlo, Chemistry and Biochemistry
Glycosylation and Phosphorylation Induce Opposing Structural Conformations in Tau's Proline-Rich Domain

LANGUAGE AND MEDIA   (ROOM 121)

Moderator: Christopher Penna, English

Taria Pritchett, English Education (McNair)
Elizabeth Higginbotham, Sociology and Criminal Justice
Essence Magazine: Tracking the Vision of Black Women

Matthew Groth, English (Humanities)
Christopher Penna, English
Shifting Attitudes in Electronic Literacy

Maria Marquez, English/Foreign Languages and Literatures (McNair)
Roberta Golinkoff, School of Education
L2 Acquisition Barriers: Considering Lexicalization Biases

Juliette Fombi, Biological Sciences (McNair)
Roberta Golinkoff, School of Education
Parental Spatial Language Production in Response to Electronic and Non-Electronic Toys

LAW   (ROOM 122)

Moderator: Eric Rise, Associate Chair, Sociology and Criminal Justice

Gabriel Mendez, Political Science (McNair)
Juliette Dee, Communication
Shaping Attitudes & Opinions Beyond the Border: A Study of Newspaper Framing on Illegal Immigration to the United States and Arizona Senate Bill 1070

Rebecca Trexler, Criminal Justice (Social Sciences)
Kenneth Haas, Sociology and Criminal Justice
Cruel Punishments, Judicial Federalism, and the Delaware Supreme Court

Alexander Holmes, Political Science (Social Sciences)
James Magee, Political Science and International Relations
Citizens United and the Future of Free Speech

Liam Phibbs, Political Science (Social Sciences)
James Magee, Political Science and International Relations
Justice Scalia: The Misapplication of Precedent

ANTHROPOLOGY   (ROOM 123)

Moderator: Karen Rosenberg, Chair, Anthropology

Jennifer Gallo, Anthropology (Social Sciences)
Peter Weil, Anthropology
Exploring Systemic Relationships between Acquaintance Rape and U.S. College Culture

Kylie Poirier, Anthropology Education (Social Sciences)
Peter Weil, Anthropology
Public Policy Reactions of Small Towns Following Rapid Latino Immigration

Daniel Reyes and Nicholas Rockwell, Anthropology (Service-Learning)
Karen Curtis, School of Urban Affairs and Public Policy
Community Partner: Food Bank of Delaware
Beating the Culture of Hunger—Rebuilding the Local Food System

Elanor Sonderman, Anthropology (Social Sciences)
Jay Custer, Anthropology
Diving Into Oyster Shell Analysis: An Exploration of the Fairlee Neck Shell Midden

Cody Prang, Anthropology (McNair)
Karen Rosenberg, Anthropology
The Evolution of the Human Foot

SOCIAL AND EDUCATIONAL SUPPORT SYSTEMS   (ROOM 124)

Moderator: Yasser Payne, Black American Studies

Deangie Davis, Psychology (McNair)
Yasser Payne, Black American Studies
No Child Left Behind: The Role of Education in Street Life Oriented Black Youth and Young Adults

Lisa Coutu, History (Humanities)
Stewart Rafert, History
Native American Education: From a Devastating Past to a Promising Future?

Stacey Chambers, Human Services (McNair)
Bahira Sherif-Trask, Human Development and Family Studies
African American Single Motherhood: The Strengths of Kinship Networks

Donors and Contributors

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Delaware Humanities Forum

Exceptional Care for Children
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Girls, Inc. of Delaware
Howard Weston Senior Center
Indigenous Pitch Dance Collective
Kuumba Academy Charter School
Nemours/Alfred I. duPont Hospital for Children
Paws for People
Red Clay School District: Richardson Park Elementary
UD Early Learning Center

Acknowledgements

Increased TGF-β1-Mediated Suppression of Growth and Motility in Castrate-Resistant Prostate Cancer Cells is Consistent With Smad2/3 Signaling

Fayth L. Miles,[1,2] Navpreet S. Tung,[3] Adam A. Aguiar,[1,2]
Senem Kurtoglu,[1,2] and Robert A. Sikes[1,2]*

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[3]*Department of Biology, Delaware State University, Dover, Delaware*

BACKGROUND. Elevated TGF-β levels are associated with prostate cancer progression. Although TGF-β is a tumor suppressor for normal epithelial and early-stage cancer cells, it may act paradoxically as a tumor promoter in more advanced cancers, although its effects are largely cell and context dependent. This study analyzed prostate cancer responses to TGF-β signaling in an isogenic model of androgen-sensitive and castration-resistant prostate cancer cells.

METHODS. Phosphorylation and nuclear translocation of Smad2 and Smad3 were analyzed using immunoblotting. Proliferation and cell cycle responses to TGF-β1 (5 ng/ml) were assessed using growth assays and flow cytometry for DNA content, as well as Western blot and immunoprecipitation of cell cycle proteins.

RESULTS. Both androgen-sensitive (LNCaP) and castration-resistant (C4-2 and C4-2B) prostate cancer cell lines demonstrated TGF-β1-induced phosphorylation and nuclear translocation of Smad2/3 that was robust in metastatic lines. Smad phosphorylation was completely abrogated with inhibition of ALK-5 kinase activity using the kinase inhibitor, SB-431542. Increased sensitivity to TGF-β1-mediated growth inhibition was observed in C4-2 and C4-2B cells, as compared to LNCaP cells. This was paralleled with downregulation of Cyclin D and increased association of p15^{Ink4b} or p27^{Kip} with CDK's. Additionally, TGF-β1 inhibited motility and invasion of metastatic cell lines.

CONCLUSIONS. TGF-β-mediated suppression of growth and motility is enhanced in metastatic, castration-resistant prostate cancer cells. Enhanced TGF-β1-induced Smad2 and -3 signaling in prostate cancer cells may correlate with tumor suppressive activity. Therefore, the direct effects of TGF-β1 on prostate cancer cells post-castration may be anti-tumorigenic and growth-suppressive. *Prostate 72:1339–1350, 2012.* © 2012 Wiley Periodicals, Inc.

KEY WORDS: TGF-β1; prostate cancer; cell cycle regulation; growth; castrate-resistant; androgen-sensitive; motility; invasion

INTRODUCTION

Prostate cancer is the second leading cause of cancer deaths in North American men [1]. The lethal phenotype of prostate cancer is the result of cytokine overload, a consequence of tumor–host interactions [2]. Various members of the transforming growth factor-beta (TGF-β) family of cytokines have been found to play an important role in prostate cancer progression. Notably, TGF-β1 levels are elevated profoundly in sera of prostate cancer patients and are associated with metastasis to bone and poor clinical outcome [3–5].

TGF-β1 is a pleiotropic cytokine involved in a variety of cellular responses including cell proliferation, apoptosis, and differentiation. In the canonical signaling pathway, TGF-β1, -2, and-3 bind to the type II receptor, TβRII, which recruits and phosphorylates the type I receptor, TβRI. This heterotetrameric receptor complex then phosphorylates Smads 2 and 3, which translocate to the nucleus to activate or repress gene transcription. A well-known tumor suppressor, TGF-β1 is a potent inhibitor of cell cycle progression, and inhibits proliferation in a variety of cell types including epithelial, endothelial, hematopoietic, and mesenchymal cells. Its inhibitory effects are exerted at the restriction point in G1 to prevent transition to S phase. Through the activity of Smad2/3, TGF-β1 may activatep21^{Cip} and p15^{Ink4b} [6–8], inhibitors of cyclin-dependent kinase (CDK) activity, and repress expression of the oncogene c-Myc, reducing the amount of c-Myc available to repress p15^{Ink4b} [9].

The central dogma of TGF-β signaling in cancer is that it exerts tumor-suppressive action on normal epithelial and early stage cancer cells, but becomes tumor-promoting for more advanced cancers, such that they undergo a switch in responsiveness to TGF-β, whereby they lose sensitivity to growth inhibition and acquire a more aggressive phenotype. Changes in TGF-β levels in the tumor or surrounding stroma in conjunction with reductions or alterations in components of the signaling pathway are associated with cancer progression and determine cellular response [10–12]. Thus, the pro-tumorigenic effects of TGF-β1 on prostate cancer could be an indirect effect of TGF-β1 signaling in the surrounding stroma. Because of the intricate network of paracrine signaling mediated by TGF-β, the direct effects of TGF-β on prostate cancer cells during progression are unclear, and the conditions surrounding and defining initiation of the diametrically opposed pro-metastatic and antiproliferative arms of TGF-β signaling are obscure. Interestingly, it is not a requirement for cancer cells to lose their growth-inhibitory response to TGF-β1 in order to form metastases [13], although it is generally more common for aggressive cancer cells to acquire resistance to its anti-proliferative activity.

Several studies have attempted to examine the ability of TGF-β to exert antiproliferative or anti-tumorigenic activity on prostate cancer cells. In the rat prostate, increased levels of TGF-β and Smad activity were reported to correlate with apoptosis post-castration [14–16]. Likewise, the aggressive, castration-resistant human prostate cancer cell line, PC-3MM2 was shown to be growth-inhibited by TGF-β, although cells overexpressing dominant negative TβRII formed significantly less metastases in nude mice [17]. Conversely, tumorigenic derivatives of

BPH1, a non-tumorigenic prostate epithelial cell line, demonstrated loss of growth inhibition to TGF-β and were induced to undergo EMT in vitro [18]. Similarly, prostate carcinoma cell lines demonstrated insensitivity to TGF-β-mediated growth suppression when compared to primary cell cultures [19]. LNCaP cells have been reported to be unresponsive to TGF-β-mediated growth inhibition due to low or undetectable levels of TGF-β receptors in the absence of DHT. Accordingly, receptor expression and sensitivity to growth-inhibition were increased in the presence of DHT [20–22]. To the contrary, it has been reported that sensitivity to growth inhibition and receptor expression are negatively regulated by DHT [23] and promoter methylation [24] in LNCaP cell lines or other prostate epithelial cell lines. Thus, the effects of this complex, pleiotropic cytokine are highly context-dependent, varied by DHT concentration, and largely a reflection of receptor functionality.

A more thorough and cohesive understanding of how TGF-β1 regulates growth and metastatic ability during progression to castrate-resistant prostate cancer may be achieved more appropriately through analysis and comparison of canonical signaling in an isogenic progression model where metastatic potential and castration-resistance increase with each derivative. Herein, we designed a study to elucidate the mechanism of TGF-β1-mediated growth inhibition and the role of Smad2/3 signaling in the cells of the LNCaP human prostate cancer progression model [25,26]. We provide evidence that elevated Smad2/3 activity is correlated with enhanced TGF-β1-mediated growth suppression and inhibition of motility in metastatic, castration-resistant prostate cancer cells. Interestingly, TGF-β1 does not alter growth or motility significantly in non-metastatic, castration-sensitive prostate cancer cells.

MATERIALS AND METHODS

Cell Culture and Reagents

The LNCaP human prostate cancer progression model consists of a series of prostate cancer cell lines derived through successive passage in castrate hosts in vivo that progress from poorly tumorigenic, non-metastatic, and androgen sensitive to metastatic and castrate-resistant. LNCaP, C4-2, and C4-2B, and PC-3 prostate cancer cells were cultured in T-medium supplemented with 5% fetal bovine serum and penicillin/streptomycin as described previously [26,27]. TGF-β1 was purchased from R&D Systems and used at a concentration of at least 5 ng/mL. The ALK-4, -5, and -7 kinase inhibitor, SB-431542, was purchased from Tocris Bioscience (Ellisville, MO) and

The Prostate

used at a concentration of 10 μM to inhibit phosphorylation of Smad2 and -3, and was added at least 30 min before TGF-β1 stimulation. Fibronectin was purchased from Sigma (St. Louis, MO) and coating was performed according to the manufacturer's instructions at 0.5 or 5 μg/cm². Growth, as measured by increased cell number, was estimated by measuring the absorbance of solubilized formazan crystals from MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide) conversion. For low- and high-density experiments 1×10^4 and 8×10^4 cells, respectively, were seeded into each well of a 24-well plate, and MTT assays was performed after 7 days. For all other MTT experiments, 1×10^5 cells were seeded into six-well plates and grown for 7 days. Cells were cultured in T-medium supplemented with 1% TCM Defined Serum Replacement obtained from MP Biomedicals, LLC (Solon, OH) in the presence of TGF-β1 or vehicle (4 mM HCL + 1 mg/ml BSA), and pre-treated with SB-431542 or 100% ETOH as indicated.

ReverseTranscription (RT)-PCR Assays

Subconfluent cells were treated with TGF-β1 or vehicle after culture for 24 hr in T-medium supplemented with 1% TCM. Cytoplasmic RNA was extracted using RNEasy Protect Mini kit (Qiagen, Valencia, CA) and cDNA synthesized from 5 μg of total RNA using Superscript III (Invitrogen, Carlsbad, CA). cDNA corresponding to 0.5 μg RNA, was used in polymerase chain reaction (PCR) with TβRII and TβRI specific, intron spanning primers: TβRI sense, 5'-CGAGTGCCAAATGAAGAGGA-3' and antisense, 5'-CGACCTTTGCCAATGCTTTC-3'; TβRII sense, 5'-ACTTTATTCTGGAAGATGCTGCT-3' and antisense, 5'-GCTGATGCCTGTCACTTGAA-3'. Actin was used as a loading control with the following primers: sense 5'-GCT CGT CGT CGA CAA CCG GCT C-3' and antisense, 5'-CAAACATGATCTGGGTCATCTTCTC-3', which generate a 353 bp product [28]. As a negative control, RNA was used in place of cDNA. The PCR products were visualized on a 1% (w/v) agarose trisacetate buffered EDTA gel.

Antibodies and Western Blot Analysis

Antibodies to phospho-Smad2 (Ser465/467), Smad2/3 (Cat. No. 3102), Smad2 (Cat. No. 3122), and p15[Ink4b] were purchased from Cell Signaling Technology (Danvers, MA) and used per manufacturer's instructions. Antibodies to phospho-Smad3 (Ser 423/Ser423, Cat. No. 04-1042), Smad3 (Cat. No. 04-1035), TβRII, and cyclin D1/2 antibody were purchased from Millipore (Billerica, MA) and used per manufacturer's instructions. Anti-TβRI-antibody was purchased from

Abgent (San Diego, CA), and Smad4 and p27[Kip] were purchased from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA) and used at a dilution of 1:500. Anti-CDK4 and Lamin B1 antibodies were purchased from Abcam (Cambridge, MA), and used at a dilution of 1:1,000. β-Actin and GAPDH antibodies were purchased from Sigma and used at a dilution of 1:5,000. For TβRII and TβRI protein analysis, subconfluent cell cultures were treated for 24 hr with TGF-β or vehicle after 24 hr of serum deprivation in T-medium with 1% TCM and lysed using modified RIPA Buffer (50 mM Tris–HCl, pH 7.4, 1% NP-40, 0.25% sodium deoxycholate, 150 mM NaCl) supplemented with protease and phosphatase inhibitors. For TβRII analysis, lysates were treated with PNGase F (New England Biolabs) before subjecting to SDS–PAGE. For TGF-β timed course experiments, subconfluent cells were lysed with nuclear RIPA (1% Nonidet P-40, 1% sodium deoxycholate, 150 mM NaCl, 10 mM sodium phosphate, pH 7.2, 2 mM EDTA, 50 mM sodium fluoride) supplemented with protease and phosphatase inhibitors. For cytosolic-nuclear fractionation, cells were first lysed with buffer A (1% DTT, 0.8% NP40 v/v) to separate cytosolic and nuclear fractions before nuclear lysis with nuclear RIPA buffer. Lysates were fractionated on SDS-acrylamide gels, and transferred onto Immobilon-P polyvinylidene fluoride or nylon-nitrocellulose (for TβRII and TβRI analysis) membranes. Membranes were blocked with TBS containing 5% milk (cyclin D1/2, p27, p15, Smad4, lamin, CDK4), PBS with 5% milk (TβRII), or TBS with 3% BSA (phospho-Smad2/3, Smad2/3, actin, and TβRI). Primary antibodies for immunoblotting were incubated overnight at 4°C, and membranes were subsequently incubated with species-specific horse-radish peroxidase (HRP)-conjugated secondary antibodies. Membranes were stripped as necessary using Restore Plus Western Blot Stripping Buffer from Thermo Scientific (Rockford, IL).

Immunoprecipitation

Subconfluent cells were lysed in "RIPA B" Buffer (1% Triton X-100, 150 mM NaCl, 5 mM EDTA, 20 mM sodium phosphate) supplemented with phosphatase and protease inhibitors, and centrifuged at 15,000 rpm for 15 min at 4°C. Two hundred and fifty micrograms of lysate was combined with 1–2 μg/ml of anti-CDK4 or anti-CDK2 antibodies (Abcam) and incubated with rotation at 4°C overnight. The lysate was subsequently combined with 25 μl of protein A agarose (Sigma) and incubated with rotation at 4°C for 60 min. Sample suspensions containing agarose beads were centrifuged at 6,000 rpm for 2 min. Pellets were washed three times in ICKA wash buffer (1%

Triton X-100, 150 mM NaCl, 10 mM sodium phosphate). Beads were resuspended in LDS sample buffer (NuPage), heated at 95°C, and subjected to SDS–PAGE. Protein was then transferred to a PVDF membrane for Western blot analysis.

Flow Cytometry Analysis

Fluorescence-activated cell sorting (FACS) was used to analyze the cell cycle phase distribution of cells after treatment with TGF-β1. Subconfluent cell cultures were grown in T-medium with 1% TCM in the presence of TGF-β1 or vehicle for 7 days. Cells were harvested with trypsin-EDTA and fixed in 70% ethanol, and treated with propidium iodide at 50 μg/ml and ribonuclease A (10 μg/ml) in 3.8 mM sodium citrate. FACS analysis was performed using a FACSCalibur (Becton Dickinson, San Jose, CA) and cell cycle profile analyzed using CellQuest software (Becton Dickinson).

Immunofluorescence and Confocal Microscopy

Subconfluent cell cultures were grown on 1.5 mm coverslips. After culture in T-medium with 1% TCM for 24 hr, cells were treated with and without TGF-β1 and SB-431542. For phospho-Smad immunostaining, cells were treated with TGF-β1, vehicle, or TGF-β1 with SB-431542 for 4 hr. Cells were fixed in 4% paraformaldehyde in TBS, washed with TBS and incubated in 1% (w/v) SDS for 10 min at 37°C, and washed. Cells were blocked in TBS with 3% BSA and 0.1% Tween (v/v) for 1 hr and incubated with anti-phospho-Smad3 (Millipore Cat No. 04-1042) in blocking buffer at 4°C overnight at a 1:100 dilution. After washing, cells were incubated with Rhodamine-conjugated anti-rabbit secondary antibody in blocking buffer at a 1:500 dilution for 1 hr at room temperature. Subsequently, cells were mounted using Vectashield containing DAPI before examination using a confocal immunofluorescence microscope. For E-cadherin and cytokeratin immunostaining, anti-E-cadherin was purchased from BD Transduction Laboratories (San Jose, CA) and polyclonal rabbit anti-cow cytokeratin was purchased from Dako (Carpinteria, CA). Cells were grown on coverslips coated with 0.5 μg/cm² fibronectin, and treated with TGF-β1 for 72 hr. Cells were washed in PBS, fixed in methanol acetone (10:1 v/v), washed, and blocked for 1 hr in PBS containing 5% normal donkey serum and 3% BSA, and incubated at a 1:250 dilution with primary antibodies. After washing, cytokeratin and E-cadherin-labeled cells were incubated for 1 hr with Rhodamine-conjugated anti-rabbit and FITC-conjugated anti-mouse secondary antibodies, respectively, at a 1:1,000 dilution. All images were acquired using an upright

Zeiss 780 confocal microscope and an apochromatic fluorescent 40× objective (Carl Zeiss MicroImaging, Thornwood, NY).

Migration Assays

For wound-healing assays, 5×10^6 cells were seeded onto 60 mm dishes coated with fibronectin at a concentration of 5 μg/cm², which was allowed to dry completely before cells were seeded. Cells were cultured in T-medium with 1% TCM for 24 hr, scratched with a silicone-coated pipette tip, and treated for 5 days with TGF-β1 or 4 mM HCl/BSA and SB-431542 or ETOH. Images were captured at treatment days 0 and 5, and later quantified by measuring migration distance in microns. For transwell (Boyden chamber) assays, 7×10^5 cells were seeded onto 8-μM pore filters coated with 5 μg/cm² fibronectin and allowed to migrate for 3 days in the presence of TGF-β1 or vehicle with or without SB-431542 treatment in T-medium containing 2.5% FBS.

RESULTS

To determine whether TGF-β receptors were expressed in the isogenic LNCaP, C4-2, and C4-2B cell lines, we performed RT-PCR on cytoplasmic RNA from subconfluent cultures using intron-spanning primers specific for TβRI and TβRII. The castrate-resistant and highly aggressive PC-3 cell line, which has been reported to express functional TGF-β receptors [20], was used as a positive control. After TGF-β1 treatment for 24 hr, increases in TβRII and TβRI mRNA were observed in LNCaP and C4-2 cells relative to actin (Fig. 1A). However, Western blot analysis showed no appreciable change in TβRII (Fig. 1B; molecular weight = 80 kDa), and a decrease in TβRI protein with TGF-β1 treatment (Fig. 1B), which was confirmed with normalization to actin (Fig. 1C). When normalized to LNCaP, TβRII levels increased with progression in the LNCaP series with an increase of nearly 1.8-fold in C4-2B cells. Conversely, TβRI levels did not change (Fig. 1D). The 80 kDa form of TβRII was undetectable in PC-3 cells, which only expressed the cleaved 60 kDa form (Fig. 1B), and protein levels in this cell line could not be compared directly to the LNCaP series due to the complete proteolytic cleavage of the receptor.

In the canonical signaling pathway, Smad phosphorylation is an indicator of functional TGF-β receptors. Thus, we examined levels of phosphorylated Smad2 (P-Smad2) in the LNCaP progression model following treatment of cells with TGF-β1 in a timed course ranging from 0 to 24 hr (Fig. 2A). Incubation with an antibody recognizing P-Smad2 and -3 at Serine 465/467 showed that Smad2 is phosphorylated in
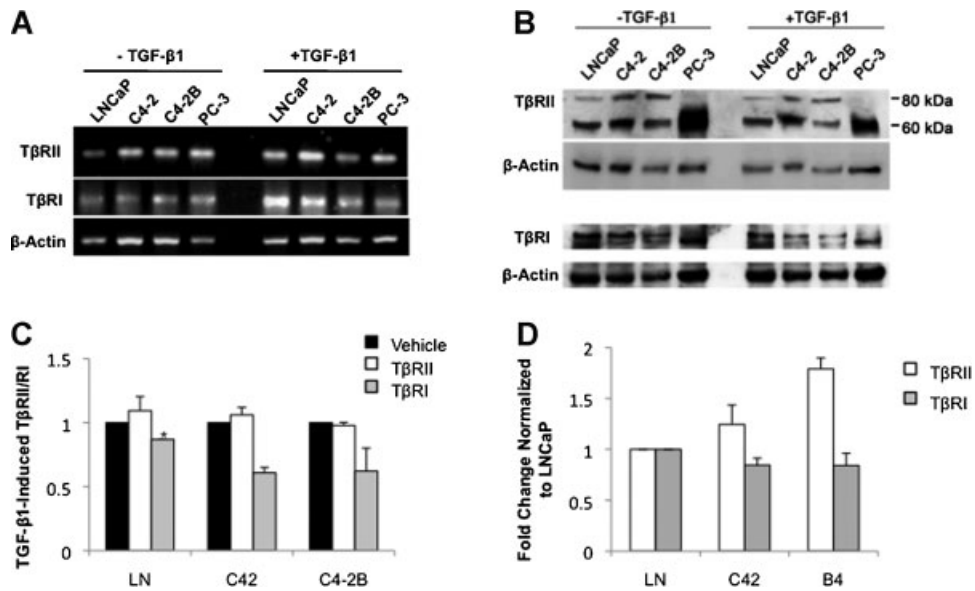
Fig. 1. Analysis of TGF-β receptor expression in cells of the LNCaP progression model. **A**: Total RNA of cells treated or untreated with TGF-β1 (10 ng/ml) was isolated and analyzed by RT-PCR for expression of TβRII and TβRI. The PC-3 cell line was used as a positive control for TGF-β receptors. **B**: Western blotting shows protein levels of TβRII and TβRI. Two predominant bands are shown—the standard 80 kDa isoform, and the cleaved 60 kDa isoform. Quantification of receptor levels shows **C**) TGF-β-induced alterations in TβRII and TβRI normalized to vehicle, and **D**) changes in receptor levels across the isogenic series. Values and error bars represent the mean fold change and range or standard error of two or more independent experiments.

LNCaP (top), C4-2 (middle), and C4-2B (bottom) cell lines by 1 hr of treatment. Incubation with anti-Smad4 showed no appreciable induction of Smad4 with TGF-β1 treatment. HeLa cell lysates stimulated with TGF-β were used as a positive control. Because total Smad2/3 levels in some cases were very difficult to detect at 0 hr, we pooled time-points treated <15 min with TGF-β1 to generate a baseline for normalization where total Smad2 levels were detectable in the absence of P-Smad2. Normalization of P-Smad2 to total Smad2 levels showed P-Smad2 was increased to nearly fourfold in C4-2 and over 2.5- and 2-fold in
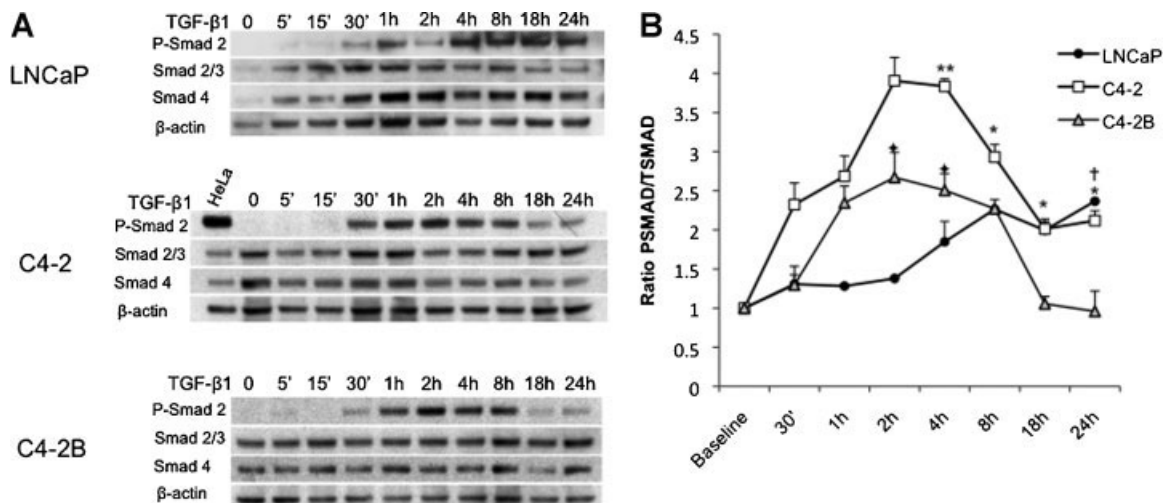
Fig. 2. TGF-β induces phosphorylation of Smad2 in LNCaP cells and metastatic sublines. **A**: Subconfluent cell cultures of LNCaP, C4-2, and C4-2B cells were treated with TGF-β1 for the indicated time periods, and whole cell lysates were subjected to SDS–PAGE for Western blot analysis of P-Smad2, total Smad2, Smad4, and β-actin. **B**: Quantification of protein levels of P-Smad2 normalized to total levels of Smad 2/3 shows TGF-β-induced increases in P-Smad2. Values and error bars represent the mean fold change and range of two independent experiments. Statistical analysis was done using Student's t-test for paired data. *, ✦, or †P-value < 0.05. **P-value < 0.005.

C4-2B and LNCaP cells, respectively. P-Smad2 levels were robust by 4 hr in all cell lines, and showed some decline by 24 hr in C4-2, which was more pronounced in C4-2B cells. However, in LNCaP cells, P-Smad2 levels were significantly elevated after 24 hr, and nearly 2.5-fold above baseline (Fig. 2B). Thus, we sought to examine nuclear presence of P-Smad2 and P-Smad3, comparing translocation after 4 and 24 hr of TGF-β1 treatment. To demonstrate that P-Smad2 and -3 are translocated to the nucleus, we performed cyto-solic-nuclear fractionation after treatment of prostate cancer cells with TGF-β1, or the combination of TGF-β1 and the ALK-4, -5, and-7 kinase inhibitor, SB-431542. Incubation with an antibody specific to P-Smad2 (Fig. 3A) or P-Smad3 (Fig. 3B) demonstrated nuclear translocation of P-Smad in LNCaP (left), C4-2 (middle), and C4-2B (right) prostate cancer cell lines. Nuclear levels of P-Smad2 and P-Smad3, to a lesser degree, showed a decline by 24 hr in C4-2, which was

less marked in C4-2B. Phosphorylated-Smad2 and -3 levels were abrogated completely with SB-431542. However, SB-431542 did not deplete total Smad2 and -3 nuclear protein levels, indicating the presence of basal levels of Smad2 and -3 in the nucleus in the presence and absence of TGF-β1 stimulation. Additionally, Smad4 did not demonstrate nuclear translocation, and nuclear Smad4 protein levels persisted even in the presence of SB-431542 (Fig. 3A and B). To visually confirm nuclear translocation, we used immunofluorescence microscopy to examine the cyto-solic-nuclear localization of P-Smad3. After treatment with TGF-β1 for 4 hr, C4-2 cells demonstrated robust nuclear staining of P-Smad3, as demonstrated with arrows (Fig. 3C). This was blocked with SB-431542 treatment, which resembled basal translocation (vehicle treatment alone). Taken together, these results indicate that Smads 2 and 3 are translocated to the nucleus after 4 hr of TGF-β1 treatment as a
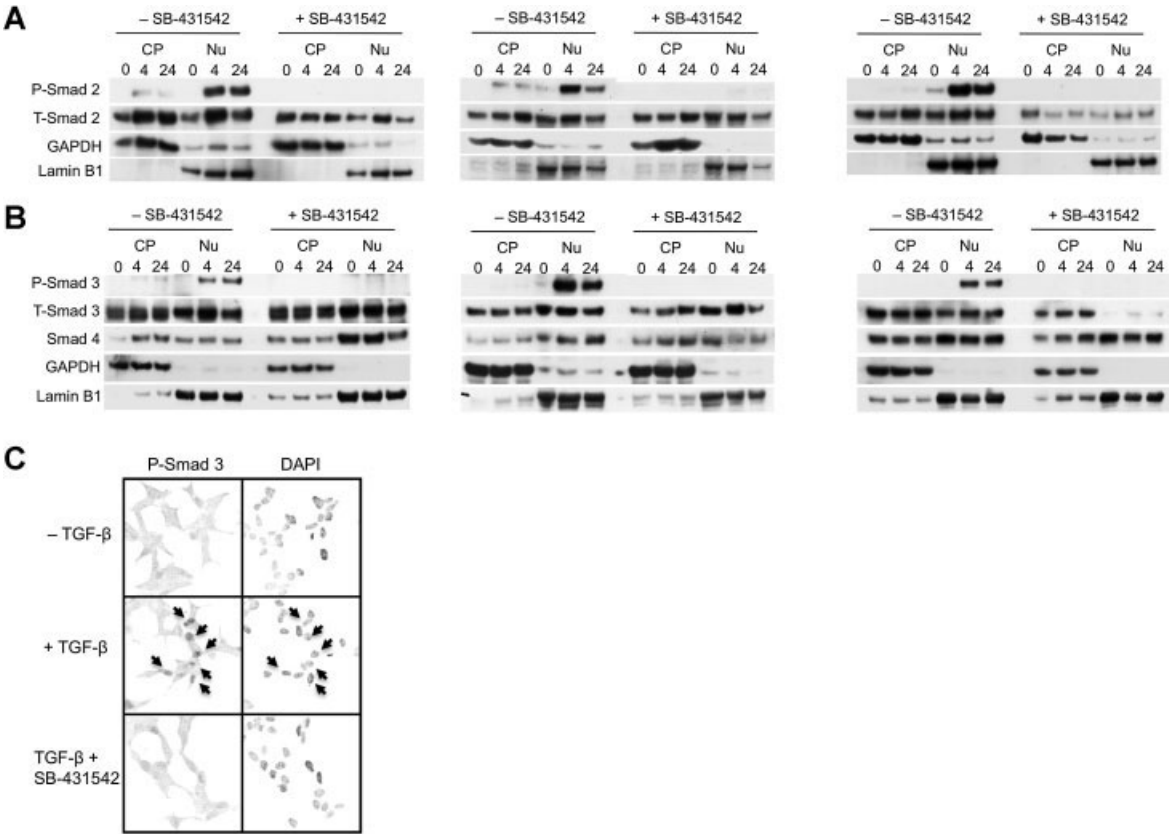
Fig. 3. TGF-β induces nuclear translocation of Smad2/3. Subconfluent cultures of prostate cancer cells were treated for 0, 4, and 24 hr with TGF-β1 after treatment with or without SB-431542. Nuclear-cytosolic fractionation was done to isolate nuclear and cytosolic proteins. Lysates were subjected to SDS–PAGE for Western blot analysis of (**A**) P-Smad2 and (**B**) P-Smad3: LNCaP (left), C4-2 (middle), and C4-2B (right). β-actin and lamin B1 were used as loading controls for cytosolic and nuclear protein, respectively. **C**: Immunofluorescence imaging was performed on C4-2 cells treated with or without TGF-β1 for 4 hr, and TGF-β in the presence of SB-431542 to examine nuclear translocation of P-Smad3. Cells were immunostained using anti-P-Smad3 (Rhodamine) and counterstained with DAPI. Arrows depict nuclei with robust localization of P-Smad3.

consequence of TβRI signaling (presumably ALK5), and Smad4 may be dispensable to Smad translocation and activation.

TGF-β1 is a known growth inhibitor in normal and early stage prostate cancer cells, and exerts its effects through Smad-mediated regulation of cell cycle proteins. We reasoned that activated Smad would correlate with a decreased growth response. We analyzed the ability of TGF-β1 to inhibit growth of low- and high-density cultures by using MTT absorbance as a surrogate for cell number. Interestingly, TGF-β1 inhibited growth most profoundly in low-density cultures, whereas growth-inhibition was much less marked at high density (Fig. 4A). LNCaP growth was the least inhibited (18%), whereas C4-2 and C4-2B cell growth was more severely inhibited (34% and 44%, respectively). Therefore, we analyzed the ability of SB-431542 to reverse TGF-β1-mediated growth inhibition of subconfluent cells. LNCaP cells were again the least inhibited (20%), whereas C4-2 and C4-2B cell growth in the presence of TGF-β1 was inhibited significantly, by 38% and 24%, respectively (Fig. 4B). SB-431542 reversed TGF-β1-mediated growth inhibition in LNCaP cells. However, in C4-2 and C4-2B cells, treatment with SB-431542 slowed overall growth, and therefore, the effects of SB-431542 on TGF-β1-mediated growth were confounded, but C4-2 cells showed partial recovery from TGF-β1-mediated growth inhibition (Fig. 4B). FACS analysis after 72 hr of TGF-β1 treatment showed that TGF-β1 did not alter the cell cycle in LNCaP (left), but reduced the relative percentage of cells in S phase in C42 (middle) and C4-2B (right) cells, although changes were modest (Fig. 4C).

To demonstrate that TGF-β1 regulates cyclins and cell cycle inhibitor proteins, we examined levels of cyclin D1/2, p15^{Ink4b}, and p27^{Kip} after TGF-β1 treatment for up to 72 hr in LNCaP (left), C4-2 (middle), and C4-2B (right) cells (Fig. 5A). As expected, we observed decreases in cyclin D1/2 levels ranging from 20% to 40%, with the greatest downregulation occurring in C4-2. Interestingly, TGF-β1 induced downregulation of total levels of p15^{Ink4b} in C4-2B cells (Fig. 5B). Given that the levels of CDKs in cells may be high under basal conditions, we undertook an analysis of the
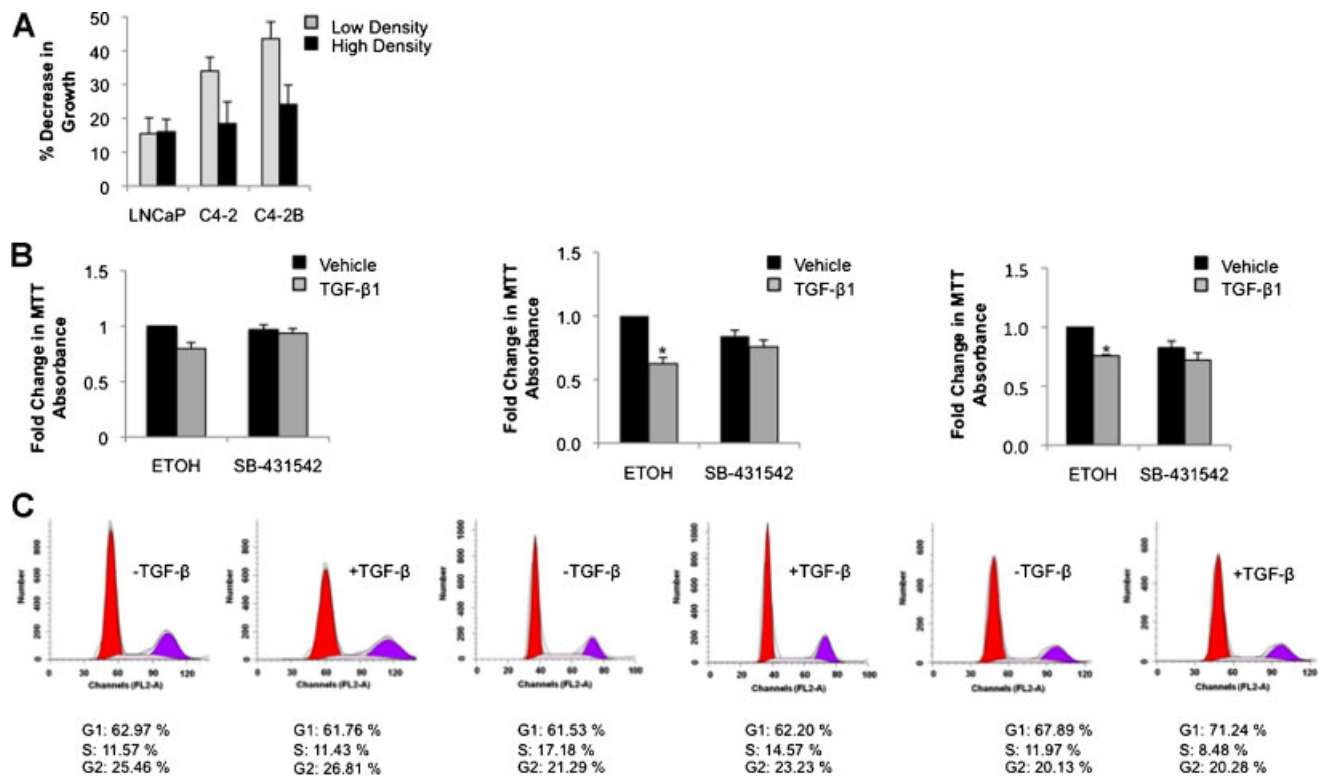
Fig. 4. TGF-β inhibits growth of metastatic LNCaP sublines. **A**: LNCaP, C4-2, and C4-2B cells seeded at low and high density were treated with TGF-β1 for 7 days before measuring MTT absorbance. The percentage of TGF-β1-induced decrease in growth at low and high density is shown. **B**: Subconfluent cell cultures of LNCaP (left), C4-2 (middle), and C4-2B (right) were treated for 7 days with vehicle (black bars) or TGF-β1 (gray bars) in the presence or absence of SB-431542, and absorbance of MTT was measured. **C**: FACS analysis was done on propidium iodide labeled samples after treatment with TGF-β1 for 72 hr to analyze cell cycle phase distribution for LNCaP (left), C4-2 (middle), and C4-2B cells (right). The represented cell cycle phases are indicated. Values and error bars represent the mean fold change and standard error of four or more independent experiments. Statistical analysis was done using Student's *t*-test for paired data. *P-value $<$ 0.05. [Color figure can be seen in the online version of this article, available at http://wileyonlinelibrary.com/journal/pros]
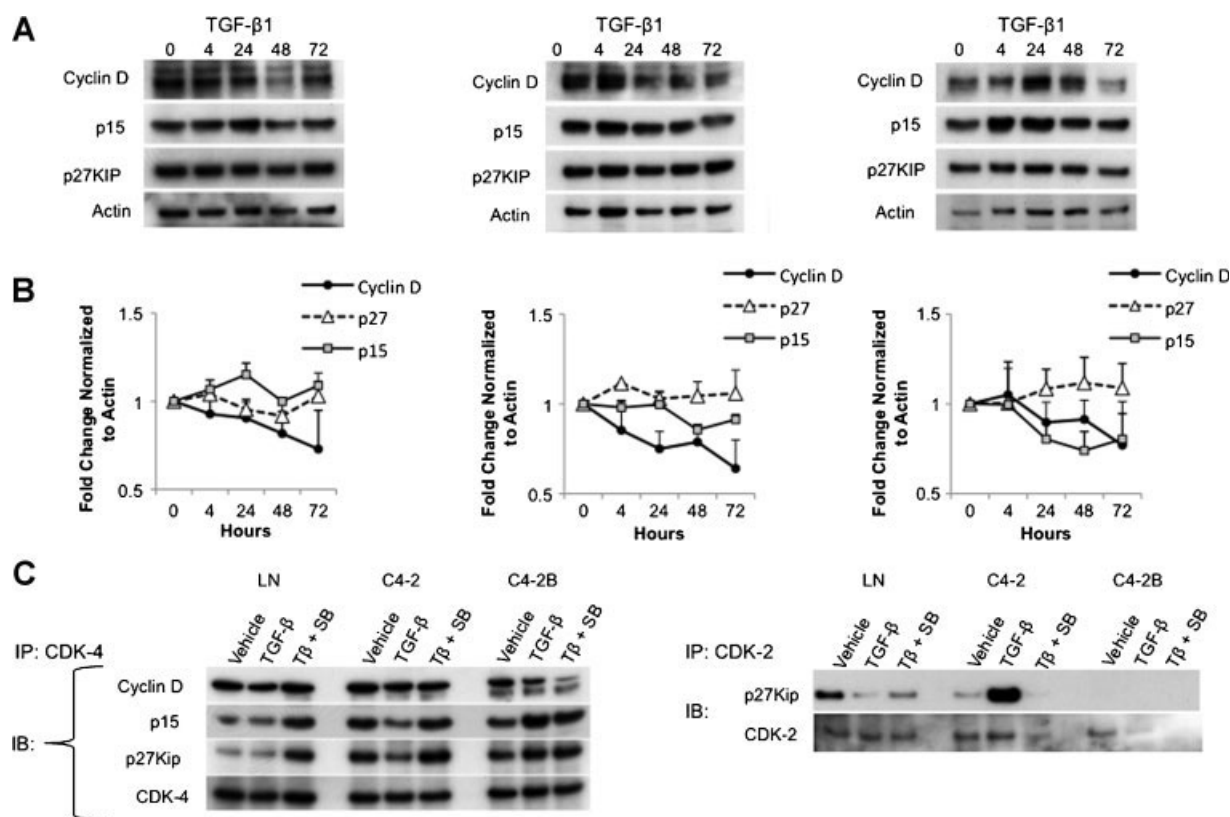
Fig. 5. TGF-β regulates cell cycle proteins. **A**: Subconfluent cell cultures of LNCaP (left), C4-2 (middle), and C4-2B (right) were treated for up to 72 hr with and without TGF-β1. Whole cell lysates were obtained as described and subjected to SDS–PAGE for Western blot analysis of Cyclin D1/2, p15^{INK 4b}, p27^{Kip}, and Actin. **B**: Protein intensity of Cyclin D, p15, and P27 was normalized to that of actin to analyze fold change in protein levels in LNCaP (left), C4-2 (middle), and C4-2B (right). **C**: Subconfluent cells treated with or without TGF-β1 for 72 hr in the presence or absence of SB-431542 were lysed as described for immunoprecipitation with anti-CDK4 or anti-CDK2 and lysates were subjected to Western blot analysis and incubated with the indicated antibodies. Values and error bars represent the mean fold change and range or standard error of two or more independent experiments.

protein complexes formed with CDKs after TGF-β1 treatment. In particular, we examined the ability of CDK4 to complex with cyclinD1/2 and the cyclin/kinase inhibitory proteins p15[Ink4b] and p27[Kip] following TGF-β1 stimulation using immunoprecipitation. Anti-CDK4 antibody was used for immunoprecipitation and subsequently immunoblotted for cyclinD1/2, p15[Ink4b] and p27[Kip]. TGF-β1 marginally downregulated the association of CDK4 and cyclin D 1/2 in metastatic cell lines and induced upregulation of CDK4-p15[Ink4b] association in C4-2B cells (Fig. 5C). However, these changes were not blocked by SB-431542. p27[Kip] showed an increase in association with CDK4 in C4-2B cells but a decrease in C4-2 cells. Interestingly, immunoprecipitation with CDK2 demonstrated increased association of p27[Kip] in C4-2 cells, suggesting that TGF-β1 suppresses growth of this cell line in a CDK2-p27[kip]-dependent manner. This association also appeared to be blocked by SB-431542, indicating that Smad2/3 may be involved in TGF-β1-

induced growth suppression, preventing transition of G_1 into S phase in C4-2 cells.

Next, we asked if TGF-β1-mediated growth-inhibition was accompanied by stimulation of motility. We reasoned that the metastatic cell lines may still respond aggressively to TGF-β1 with increased migration in spite of growth-inhibition. After treatment with TGF-β1 or vehicle for 7 days in a wound-healing assay, migration in the presence of TGF-β1 was unchanged in LNCaP (left) and inhibited in C4-2 (middle), and C4-2B (right) by 58% and 45%, respectively (Fig. 6A and B). SB-431542 completely reversed TGF-β1-regulation of migration of C4-2 and C4-2B and marginally stimulated motility of LNCaP. To confirm further the ability of TGF-β1 to inhibit motility, we examined invasion in the presence of TGF-β or SB-435142 after 72 hr in a Boyden chamber in LNCaP (left) and C4-2 (right) cell lines (Fig. 6C). As expected, C4-2 cells were more inhibited by TGF-β1 than LNCaP, but SB-431542 did not reverse the TGF-β1-
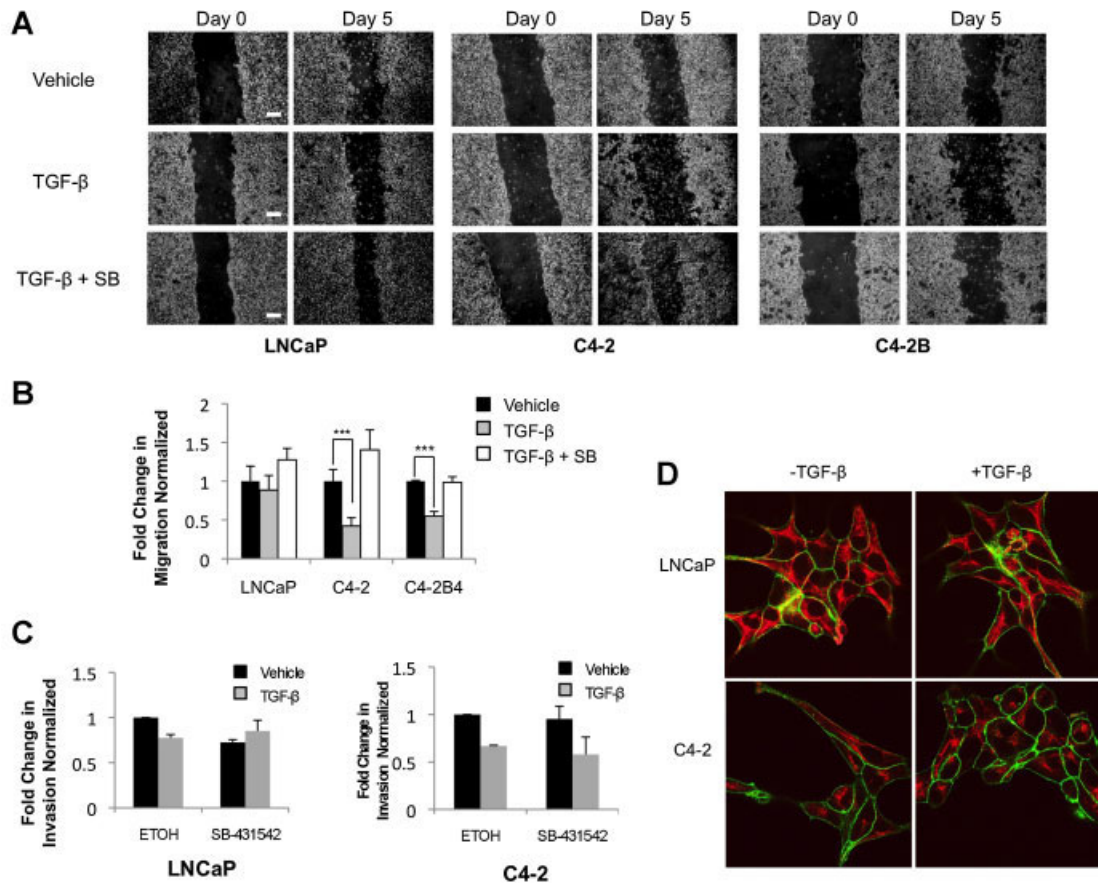
Fig. 6. TGF-β1 suppresses motility on fibronectin. **A**: LNCaP, C4-2, and C4-2B cells were plated at high density onto fibronectin-coated dishes and scratched to induce a wound. Cells were treated for 5 days with or without TGF-β1 and SB-431542. Representative phase microscopy images are shown. Scale bar represents 250 μm. **B**: Distance migrated was analyzed and normalized to vehicle control. **C**: 7.5×10^4 LNCaP (left) or C4-2 (right) cells were seeded onto 8-μm fibronectin-coated filters of a Boyden chamber, and allowed to migrate towards a TGF-β1 gradient for 3 days in the presence or absence of SB-431542. **D**: Subconfluent cultures of LNCaP and C4-2 cells were treated for 72 hr with TGF-β or vehicle and immunostained with antibodies to E-cadherin (FITC) and cytokeratin (Rhodamine). Immunofluorescence imaging was performed using confocal microscopy to capture changes in the distribution and levels of E-cadherin and cytokeratin. Values and error bars represent the mean fold change and range or standard error of two or three independent experiments. Statistical analysis was done using Student's t-test for paired data. ***P-value $= 0.005$.

mediated inhibition of invasion in C4-2 cells, suggesting that ALK-5 is not required for inhibition of invasion.

As TGF-β is a well-known inducer of EMT in various cancer cell types, including prostate, we wanted to determine whether TGF-β1 maintained the ability to induce EMT in our castration-resistant prostate cancer cells. We used confocal microscopy to examine the distribution of E-cadherin and cytokeratin. After 72 hr of TGF-β1 treatment, E-cadherin (FITC-labeled) was unchanged compared to control in both LNCaP and C4-2 cells, and cytokeratin (Rhodamine-labeled) remained high in the presence and absence of TGF-β1 (Fig. 6D). These data indicate the lack of induction of an epithelial to mesenchymal phenotype with TGF-β1 stimulation.

DISCUSSION

Here, we have shown that TGF-β1 inhibits growth and metastatic behavior in castrate-resistant prostate cancer cell lines, and these events appear to be mediated, at least in part, by Smad2/3 signaling. Importantly, we have found that TGF-β1 induces association of functional CDK4-p15 and CDK2-p27[Kip]-complexes, without upregulating total levels of CDK inhibitors. Unlike the metastatic C4-2 and C4-2B sublines, the parental LNCaP cell line is sensitive only marginally to TGF-β1-mediated suppression of growth, and motility is unaffected by TGF-β1. Interestingly, however, all cell lines express functional TβRII and TβRI receptors, and phosphorylate Smad2 and -3. Our data show a trend towards increased

levels of TβRII in the metastatic and castration-resistant prostate cancer cell lines compared to the androgen-sensitive, non-metastatic LNCaP cells. This increase in receptor levels correlates with increased sensitivity to TGF-β1-mediated growth suppression (Fig. 4). Although tissue staining of prostate cancer specimens has revealed loss or decline in TGF-β receptor levels, which correlates with progression [29–32], the majority of prostate cancers maintain TGF-β receptors, and therefore, the observation of detectable TGF-β receptors in our prostate cancer cell lines is consistent with the general receptor status of human prostate cancer.

The ability of SB-431542 to abrogate consistently phosphorylated Smad levels indicates a complete inhibition of TβRI kinase activity, and a typical role for TβRI in canonical Smad signaling after TGF-β1 stimulation. Total Smad levels, including nuclear protein, were not abrogated with SB-431542 (Fig. 3A and 3B) except in C4-2B where the effect was not dramatic. This can be expected as non-phosphorylated Smad frequently is distributed in both the cytosol and nucleus under basal conditions. However, while inhibition of TβRI kinase activity reversed some effects of TGF-β1 on prostate cancer cells, results were confounded by the effect of SB-431542 on overall cell growth. This slower growth response observed with SB-431542 treatment (Fig. 4B) may signify the presence of autocrine growth factor activity acting through an SB-431542-inhibited TβRI, namely ALK-4, -5, or -7. Indeed, this possibility is strengthened with the findings of decreased levels of Cyclin D and elevated levels of CDK inhibitors in the presence of SB-431542, independent of TGF-β1-induced changes alone (Fig. 5C). While Smad2/3 likely mediates inhibition of lateral motility, our data suggest that Smad2/3 is not involved fundamentally in TGF-β1-mediated downregulation of invasion (Fig. 6C). Collectively, the data infer the existence of separate mechanisms of inhibition of lateral and directional motility.

Our data imply an unconventional role for P-Smad2 and P-Smad3 in mediating TGF-β1-induced transcriptional activity independent of Smad4 transactivation. This is supported by the lack of nuclear translocation and enrichment of Smad4 in the presence of TGF-β1 in parallel with P-Smad2 and P-Smad3 (Fig. 3), although additional studies are necessary. The different responses observed in the non-metastatic and metastatic prostate cancer cells highlight the complex roles of Smad transcription factors, and suggest a compound role for Smads in prostate cancer cells. Typical growth-inhibiting Smad2/3 activity in the metastatic lines is contrasted with a less inhibited and more ambiguous response in

LNCaP cells. Additionally, the sustained phosphorylation of Smad2 in LNCaP cells is contrasted with somewhat diminished phosphorylation in metastatic sublines. These are novel findings in a linear model of increasingly metastatic and castrate-resistant prostate cancer cells. Such an observation might be explained in the context of the myriad of Smad cofactors that mediate transcription [33], in conjunction with the absence of paracrine stromal cell signals. Differences in Smad phosphorylation and activity have critical implications for disease progression and castration-resistance in the context of a TGF-β-rich environment.

The inability of TGF-β1 to induce downregulation of E-cadherin or cytokeratin indicates that TGF-β does not induce EMT of LNCaP and metastatic sublines after 72 hr under our culture conditions. Although this is contrary to other reports of TGF-β-induced EMT in epithelial and cancer cell lines, this observation correlates with our findings of downregulated motility and the absence of an invasive phenotype with TGF-β stimulation (Fig. 6).

Our findings elucidate the tumor- and motility-suppressing roles of TGF-β in advanced prostate cancer cells—an observation that is contrary to other findings that prostate cancer cells become resistant to the growth-inhibitory effects of TGF-β while demonstrating increased motility [17,34,35], along with the general dogma of TGF-β signaling in cancer. The fact that our experiments were performed in the context of the epithelial cells, reflecting only tumor cell autonomous interactions may explain this apparent paradox, in part. The relevance of the stromal environment, which largely modulates cellular responses to TGF-β cannot be ignored, as the dynamic tumor-stromal cell interplay ultimately dictates disease progression. In fact, it has been shown that loss of TβRII in prostatic stroma leads to PIN lesions and adenocarcinoma [36,37]. Differences in TGF-β responses in the presence of stromal cells reflect further the diversity of TGF-β signaling. It is likely that TGF-β exerts pro-metastatic effects indirectly on prostate cancer cells through stromal cells, while its direct effects on epithelium are anti-proliferative. Because bone provides the microenvironment where prostate cancer cells ultimately colonize, our future studies will examine the contribution of the bone microenvironment to TGF-β-regulated growth and metastatic behavior. The study of TGF-β-regulated events in a linear progression model in light of bone marrow stromal cell interactions will be an invaluable insight in the understanding of the ''switch'' to an aggressive, uninhibited phenotype, and may highlight the disparities of TGF-β signaling during tumor cell autonomous and tumor–host interactions.

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Research Article

Individual Rac GTPases Mediate Aspects of Prostate Cancer Cell and Bone Marrow Endothelial Cell Interactions

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The Rho GTPases organize the actin cytoskeleton and are involved in cancer metastasis. Previously, we demonstrated that RhoC GTPase was required for PC-3 prostate cancer cell invasion. Targeted down-regulation of RhoC led to sustained activation of Rac1 GTPase and morphological, molecular and phenotypic changes reminiscent of epithelial to mesenchymal transition. We also reported that Rac1 is required for PC-3 cell diapedesis across a bone marrow endothelial cell layer. In the current study, we queried whether Rac3 and RhoG GTPases also have a role in prostate tumor cell diapedesis. Using specific siRNAs we demonstrate roles for each protein in PC-3 and C4-2 cell adhesion and diapedesis. We have shown that the chemokine CCL2 induces tumor cell diapedesis via Rac1 activation. Here we find that RhoG partially contributes to CCL2-induced tumor cell diapedesis. We also find that Rac1 GTPase mediates tight binding of prostate cancer cells to bone marrow endothelial cells and promotes retraction of endothelial cells required for tumor cell diapedesis. Finally, Rac1 leads to $\beta1$ integrin activation, suggesting a mechanism that Rac1 can mediate tight binding with endothelial cells. Together, our data suggest that Rac1 GTPase is key mediator of prostate cancer cell-bone marrow endothelial cell interactions.

1. Introduction

Skeletal metastases represent a major clinical problem for men suffering from prostate cancer (PCa). Nearly 80% of men who die from this disease have significant spread of the cancer to bone [1, 2]. Like all cancers, PCa cells must successfully complete a series of ordered steps, known as the metastatic cascade, to form a distant tumor [3, 4]. One key step in the PCa metastatic cascade is the process of extravasation from the circulation into the bone microenvironment [5, 6]. The process of PCa cell extravasation can be subdivided into a number of substeps, which include arrest, binding, adhesion, and spreading on bone marrow endothelial cells, migration along the endothelial barrier, tumor cell diapedesis, and invasion into the bone stromal compartment [6–8]. Although many of these substeps have

been well studied for leukocyte extravasation, relatively little is known about the process of PCa tumor cell extravasation across a bone marrow endothelium (reviewed in [9]).

The Rho GTPases are a group of proteins that comprise a subfamily of the Ras-superfamily of monomeric GTP-binding proteins that act as molecular switches regulating the cytoskeleton promoting cell migration [10–14]. Furthermore, the Rho proteins are implicated in cancer progression and metastasis (reviewed in [15]). Previously, we have suggested potential roles for individual Rho GTPases in PCa extravasation [16–18]. Specifically, we demonstrated that RhoC GTPase is required for invasion in response to insulin-like growth factor I and type I collagen [16, 18]. Upregulation of the integrin heterodimer $\alpha2\beta1$ in LNCaP cells selected for their ability to bind to type I collagen led to increased RhoC activation and cellular invasion upon

integrin ligation [19, 20]. Downregulation of RhoC in PC-3 human PCa cells through introduction of either a dominant negative (dn)RhoC or a RhoC-specific shRNA led to a significant decrease in the cells ability to invade either collagen or Matrigel-coated filters [16, 18]. However, these cells underwent changes reminiscent of epithelial to mesenchymal transition (EMT). Concordant with EMT, the cells displayed increased random linear motility, which was due to increased and sustained levels of Rac1 GTPase expression and activation. Further, we demonstrated that active Rac1 GTPase is required for PC-3 cell diapedesis across a BMEC layer [18].

The Rac GTPase branch of the Rho subfamily is comprised of four members, Rac1, Rac2, Rac3, and RhoG. Rac1 and RhoG are ubiquitously expressed, while Rac2 is primarily expressed in hematopoietic cells, and Rac3 is expressed mainly in nervous tissue but can be found expressed at lower levels in most other tissues (reviewed in [21]). Seminal experiments demonstrated a role for Rac1 in the formation of lamellipodia [11, 13, 14, 22, 23]. Recent evidence suggests a role for Rac3 GTPase in cellular adhesion and neurite outgrowth [24–26]. RhoG GTPase has been shown to signal in a parallel pathway to Rac1, being regulated by some of the same upstream regulatory proteins such as Vav2 and activating some of the same downstream effectors as Rac1 [27]. RhoG has also been shown to act as a hierarchical GTPase; activation of RhoG can lead to the activation of Rac1 through direct interaction with the Dock180-ELMO [28]. Therefore, Rac1-mediated cell migration can be regulated through direct activation of RacGEFs or via RhoG GTPase [29].

Rac1 GTPase plays an intimate role in monocyte and macrophage diapedesis [30]. Monocytes are recruited to the sites of inflammation via stimulation by the chemokine CCL2 (a.k.a. MCP-1) [31]. Binding of CCL2 to its putative receptor CCR2 leads to clustering of the novel actin regulatory protein PCNT1 to the cells leading edge and regulation of migration through activation of Rac1 [30]. Activation of Rac1 is required to form lamellipodia, which in turn is required for monocytes to sense junctions between endothelial cells [30]. BMECs from PCa patients secrete high levels of CCL2 [32]. Stimulation of PC-3 cells with CCL2 results in activation of Rac1 GTPase and EMT consistent with what is observed when RhoC activity is downregulated through introduction of a dnRhoC or shRNA to RhoC [16–18]. Furthermore, CCL2 stimulation drives PC-3 tumor cell diapedesis across a BMEC layer via PCNT1 [17].

Levels of Rac1 and Rac3, but not Rac2, are shown to be increased in prostate cancer patient samples compared to normal prostate [33]. However, the role that these GTPases play in PCa tumor cell metastasis has not been thoroughly studied. Similarly, there is no information on the contribution of RhoG to PCa progression. In the current study we investigate the roles of Rac1, Rac3, and RhoG GTPases in the process of prostate tumor cell diapedesis across a bone marrow endothelial cell layer. All three Rac proteins have an influence on tumor cell diapedesis across a bone marrow endothelial cell monolayer. Further, we demonstrate that Rac1 GTPase has a significant effect on PCa cell diapedesis,

while Rac3 has a negative effect on tumor cell diapedesis. In addition, RhoG has a partial effect on CCL2-stimulated diapedesis. Finally, Rac1 regulates binding of prostate cancer cells to the bone marrow endothelial cells. Our data suggest that Rac1 is required for the activation of β1 integrins leading to binding of the prostate cancer cell to the BMEC. This is the first study to demonstrate roles for different isoforms of Rac GTPase in the PCa metastatic phenotype.

2. Materials and Methods

2.1. Cell Lines and Cell Culture. PC-3 PCa cell lines were obtained from American Type Culture Collection (Manassas, Va) and maintained in Ham's F-12 medium with 1.5 g/L sodium pyruvate, 2 mM L-glutamine, and 10% FBS (Invitrogen/Gibco, Carlsbad, Calif). C4-2 cells were a gift from Dr. Robert Sikes (University of Delaware) and maintained in T-medium containing 10% FBS (Invitrogen/Gibco). Human bone marrow endothelial cells (BMECs) were a gift from Dr. Graca Almeida-Porada (University of Nevada School of Medicine, Reno, Nevada). Cultures were maintained in Medium 199 with Earles's salts, L-glutamine, 2,200 mg/L sodium bicarbonate, 25 mM HEPES (Invitrogen/Gibco) buffer, 10% FBS, 1% pen/strep, endothelial cell growth supplement (BD Biosciences, Bedford, Mass), and 7500 u/500 mL media of heparin (Sigma-Aldrich, St. Louis, Mo). All cell lines were maintained at 37°C in a 90% : 10% air : CO$_2$ incubator. C3 exotransferase was introduced into cells as previously described using a lipid transfer-mediated method [34] and treated for 2 h before analysis. Rac1 inhibitor NSC23766 (Calbiochem, San Diego, Calif) treatment was performed by adding directly to tissue culture medium to a final concentration of 100 μM 1 h prior to analysis. Prostate cancer cells were stimulated with 100 ng/mL recombinant human (rh)CCL2 (MCP-1) in tissue culture medium (Millipore-Chemicon Inc., Billeceria, Mass) for 30 min during the Rac activation assays and kept in the presence of the chemokines during the diapedesis assays.

2.2. siRNAs. Specific siRNAs for human Rac1 and Rac3 GTPases were a gift from Dr. Marc Symons and described previously [26]. RhoG siRNA and scrambled control siRNAs were synthesized by integrated DNA technologies. RhoG siRNA target sequences were (1) 5′-TGCCCTGATGTG-CCCATCCTGCTGGTGGG-3′ and (2) 5′-ACGTGCCTG-CTCATCTGCTACACAACTAA-3′. The Rac1, Rac3, and RhoG siRNA duplexes were formed by adding 30 μL of each RNA oligo solution together with 15 μL of 5x annealing buffer (100 mM NaCl and 50 mM Tris-HCl pH 7.5) to give a final volume of 75 μL and a final concentration of 20 μM; incubated for 2 min in water bath at 95°C; allowed to cool to room temperature. Additional experiments were performed using ON-TARGET plus SMARTpool siRNAs Rac1, Rac3, and RhoG siRNAs that were obtained from Dharmacon (Dharmacon/Thermo Scientific, Layfette, Colo). siRNAs were transfected into prostate cancer cells using FuGene6 (Roche, Indianapolis, Ind) or GeneSilencer Reagent (Genlantis, San Diego, Calif) per the manufacturers instructions

and cells used 72 h after transfection. For rescue experiments, mutations were generated using the QuickChange II Site-Directed Mutagenesis kit (Stratagene) according to the manufactures recommendations. To fully abolish the effect of siRNAs, two nucleotides in the siRNA-targeted area were changed in both Rac3 and RhoG GTPases. To create a RhoG fast cycling mutant, glutamine 63 was converted to lysine.

2.3. Reverse Transcriptase and Real-Time Quantitative PCR. Total RNA was harvested from cells and converted to cDNA as previously described [16]. PCR primers were designed using the primer design feature on the Evocycler PCR program (Evogen Ltd., UK). Primer design parameters were set to optimally produce PCR products between 100 and 150 bp in size. Primer sequences are found in Supplemental Table 1 (see Table 1 in Supplementary Material available online at doi:10.1155/2011/541851). RT-PCR was performed on an Evocycler EPx (Evogen Ltd.) using Fast SYBR Green chemistry (Applied Biosystems Inc., Foster City, Calif) per the manufacturers recommendations for 30 cycles (98°C for 15 s, 67°C for 15 s, and 72°C for 30 s), and PCR products visualized on a virtual gel and band intensities were normalized to GAPDH using the Evocycler PCR program.

For quantitative (q)PCR, RNA was isolated from the cell lines using TRIzol Reagent (Invitrogen, Carlsbad, Calif). cDNA was synthesized from this RNA using the Promega Reverse Transcription kit (Promega Corp., Madison, Wis). Appropriate primers (Integrated DNA Technologies, Inc., Coralville, Iowa) were diluted to a final concentration of $10 \mu M$. The cDNA synthesized from the isolated RNA was diluted to a final concentration of $4 ng/\mu L$. Reactions were prepared as a bulk "master mix" using the ABI SYBR Green PCR Master Mix (Applied Biosystems Inc., Foster City, Calif) for each target gene/primer pair used. Three no-template controls were included for each primer pair being used. A $5 \mu L$ aliquot of cDNA was pipetted into each well of the ABI 96-well plate, and $20 \mu L$ of the reaction master mix was added to it. Plates were covered with ABI adhesive cover, centrifuged at 1000 rpm to mix the contents, and run on an ABI 7000 real-time qPCR machine housed in the Center for Translational Cancer Research (University of Delaware).

2.4. Tumor Cell Diapedesis Assays. Tumor cell diapedesis assays were performed as previously described [18]. Briefly, 100,000 HBME cells were added to the top chamber of either uncoated or Matrigel-coated Transwells 24 h prior to the assay and allowed to form a confluent monolayer. PC-3 and C4-2 cells were harvested, labeled with Calcein AM (Invitrogen/Molecular Probes) per manufacturers recommendations, and resuspended in serum-free medium containing 0.1% BSA at a concentration of 3.75×10^5 cells/mL, and 0.5 mL was added to the top chambers. The chambers were incubated for 24 h at 37°C in a 10% CO_2 incubator. Medium was aspirated from the top chamber, and excess Matrigel and cells were removed from the filter using a cotton swab. Filters were cut away from the inserts, mounted on microscope slides, and visualized on a fluorescent microscope and number of invaded cells counted.

2.5. Rac GTPase Activation Assay. Activation of total Rac GTPase proteins was performed using a GLISA pan-Rac activation assay kit (Cytoskeleton Inc., Denver, Colo) as previously described [18]. Briefly, prostate cancer cells were grown to 75% confluence in a 100 mm dishes and serum starved for 24 h. On the day of the assay, cells were harvested using nonenzymatic cell dissociation buffer (Sigma-Aldrich), washed twice with ice-cold PBS, and resuspended in $65 \mu L$ GLISA lysis buffer. Protein lysates were transferred to ice-cold 1.5 mL centrifuge tubes and clarified by centrifugation at 10,000 rpm for 2 min. Protein concentrations were determined using the supplied Precision Red advance protein assay and 1.0 mg/mL protein used for the GTPase activation assay per manufacturers recommendations. After antibody and horseradish peroxidase detection reagent incubation, signals were detected on a Benchmark Plus microplate spectrophotometer at 490 nm (Bio-Rad Laboratories, Hercules, Calif).

2.6. Atomic Force Microscopy. All AFM experiments were conducted with a Bioscope II (Vecco, Santa Barbara, Calif) using silicon-nitride tips (Vecco; spring constant 0.06 N/m). Unbinding force measurements were conducted with tips functionalized with collagen or fibronectin (Becton-Dickinson, Franklin Lakes, NJ) at concentrations of $50 \mu g/mL$ and $15 \mu g/mL$, respectively. Likewise, 35 mm tissue culture dishes (Corning Inc., Corning, NY) were coated with collagen or fibronectin and sterilized under ultraviolet light overnight. PC-3 cells were transfected with siRNA specific for Rac1, Rac3, or RhoG using FuGene6 (Roche) or GeneSilencer Reagent (Genlantis) and plated on the prepared dishes 8 h prior to experimentation. BMECs were cultured in RPMI 1640 media (Hyclone/Thermo Scientific) supplemented with 10% FBS. The functionalized AFM tip was dropped onto a single live BMEC cell and after attachment was verified, the loaded tip was gently lowered onto the center of a PC-3 cell. The unbinding force interaction between the two live cells was measured. The unbinding force is the force required to separate two adhesion molecules and is measured in picoNewtons (pN). The number of events for a particular unbinding force is the number of molecules separated at each force. Specifically, 250 unbinding events were captured per cell site with 4 areas probed per cell, and 3 separate cells were probed per treatment. Force curves were generated at a frequency of 1 Hz in a relative trigger mode.

AFM stiffness measurements were based on recording the elastic response of cells, BMECs and PC-3s using an AFM tip. The AFM was operated in the force-volume mode for recording a set of loading/unloading load displacement curves at a frequency of 1.03 Hz and a forward/reverse velocity of $4.11 \mu m/sec$. The resultant measurement is the dynamic elastic modulus (a.k.a. the Young's modulus), which measures the stiffness of the cell. The Young's modulus is the ratio of stress to strain and is thus represented by units of pressure, Pascals (Pa). Cell stiffness changes are due to morphologic changes resulting from alterations in cytoskeletal structure (reviewed in [35]). The elastic modulus was measured with individual BMECs, individual PC-3 cells, and

the duo: PC-3 cells attached to plated BMECs and BMECs attached to plated PC-3 cells. The elastic modulus for the BMEC/PC-3 combinations was generated for the plated cell, and the attached cells separately. Each force-volume map consists of 256 data points per sample site with 3 separate sites measured per experimental condition, 3 separate times.

2.7. Transendothelial Electrical Resistance (TEER). Transendothelial electrical resistance (TEER) measurements were done using Epithelial Voltohmmeter (EVOM; World Precision Instruments Inc., Sarasota, Fla) following manufacturers directions. Briefly, BMECs were plated at a concentration of 1.3×10^6 cells/mL on 12-well $0.4\,\mu$ polycarbonate membrane inserts (CLS3401; Corning Transwell) and were maintained until day 4 (we determined empirically that the TEER for the BMEC monolayer was optimum on day 4 after plating due to maturation of cell junctions). On day 4, tissue culture medium was removed from the top chamber, an equal concentration of PC-3 cells was added to the BMEC monolayer and TEER measured at specified intervals.

2.8. Fluorescence-Activated Cell Sorting (FACS) Analysis. Prostate cancer cells were cultured in T25 flasks (Corning Inc., Edison, NJ), detached, washed, and resuspended in 5% bovine serum albumin (BSA; Sigma-Aldrich) in phosphate buffered saline (PBS; Sigma-Aldrich). All washes and resuspensions were also performed in 5% BSA containing PBS. One set of control and siRac1-transfected prostate cancer cells were each further treated with CCL2 (100 ng/mL) for 30 min, washed, and resuspended. The several states of $\beta 1$ activation were queried with two conformation-sensitive antibodies N29 (BD Biosciences, Franklin Lakes, NJ) and HUTS-21 (BD Biosciences) in addition toa total $\beta 1$ conformation-insensitive antibody, MAR4 (Chemicon, Billerica, Mass). All antibodies were used at a final concentration of $10\,\mu$g/mL, and all incubations were conducted in the dark and at 37°C. Cells were analyzed using an FACS Calibur cytometer (BD Biosciences), equipped with 488 nm and 633 nm lasers. Analyses were performed on 10,000-gated events, and the numeric data were processed with Cellquest software (Becton Dickinson).

2.9. Statistical Analysis. All experiments were performed a minimum of three separate times with individual transfections consisting of no less than three replicates per experiment. Statistical analysis of the combine experiments was performed using GraphPad Prism and by the University of Delaware College of Agriculture and Natural Resources Statistics Laboratory. A one-way ANOVA analysis was used with Bonferroni's post hoc analysis for comparison between multiple groups. A Students t-test was used for comparison between two groups. Significance was defined as a P value < .001. Data is represented as mean ± standard deviation.

3. Results

3.1. Active Rac GTPases Affect Prostate Cancer Cell Transendothelial Migration. Previously, we demonstrated that Rac1

GTPase was required for tumor cell transendothelial cell migration. However, we did not thoroughly explore if other Rac family members contributed to diapedesis [16–18]. The expression of Rac1 and Rac3 GTPases is increased in PCa patient tumors; however, it is unknown if RhoG is expressed [33]. Using quantitative (q)PCR, we demonstrate detectable message for Rac1, Rac3 and RhoG in the PC-3 cells. As shown in Figure 1(a), normalized Rac1 mRNA expression levels were on average 8-fold higher than both Rac3, and RhoG, suggesting that Rac1 is the predominant Rac GTPase expressed in the PC-3 cells. Expression levels were confirmed when the products of a semiquantitative PCR were visualized by virtual gel (Supplemental Figure 1). Band intensity for each product on the virtual gel is automatically normalized to the corresponding GAPDH. Similar expression levels were observed for C4-2 prostate cancer cells (Supplemental Figure 2(a)). Due to the lack of specific antibodies, particularly for Rac3, protein expression levels were not assessed by Western blot analysis.

To elucidate the role of each Rac protein in transendothelial cell migration, we selectively downregulated the expression of Rac GTPase isoforms using siRNA. Figure 1(a) is the results of isoform-specific Rac message depletion using siRNA duplexes. Expression of each Rac mRNA was significantly reduced by a minimum of 80% compared to PC-3 cells treated with an appropriate scrambled control. Each siRNA specifically reduced its target without affecting other Rac GTPases or affecting cell growth (growth data not shown). Similar results were seen when alternate siRNAs were used for each Rac isoform.

PC-3 cells were treated with the pharmacologic RacGEF inhibitor NSC23766 (iRac) or Rac-specific siRNA and the effect on total Rac activity determined (Figure 1(b)). As expected, both the NSC23766 inhibitor and Rac1-specific siRNA reduced total active Rac levels by ~60% compared to untransfected control. Interestingly, knockdown of RhoG led to a significant 45% reduction in total Rac activity suggesting that RhoG may activate Rac1 during physiologic process such as diapedesis. In contrast to Rac1 and RhoG, knockdown of Rac3 resulted in a significant 52% increase in total Rac activity compared to control.

Since Rac GTPases are required for transendothelial cell migration across a BMEC layer [18], we next tested the individual role of Rac1, Rac3, and RhoG in PCa diapedesis across a BMEC layer. As expected, downregulation of Rac1 led to a significant decrease in diapedesis (Figure 1(c)). However, inhibition of RhoG and Rac3 had no effect on inhibiting tumor cell diapedesis. In contrast to Rac1, depletion of Rac3 led to a 70% increase in transendothelial migration, suggesting that Rac3 limits PCa diapedesis similar to what has been shown for RhoA in PCa invasion [18]. The increase in diapedesis observed when RhoG was depleted approached but did not achieve significance compared to untransfected or scrambled controls. Figure 1(d) demonstrates that expression of a siRNA-resistant Rac3 in Rac3-downregulated cells results in a significant decrease in diapedesis. Similarly, re-expression of RhoG led to a significant decrease in diapedesis compared to cells depleted of RhoG. This suggests a negative effect of Rac3 and possibly
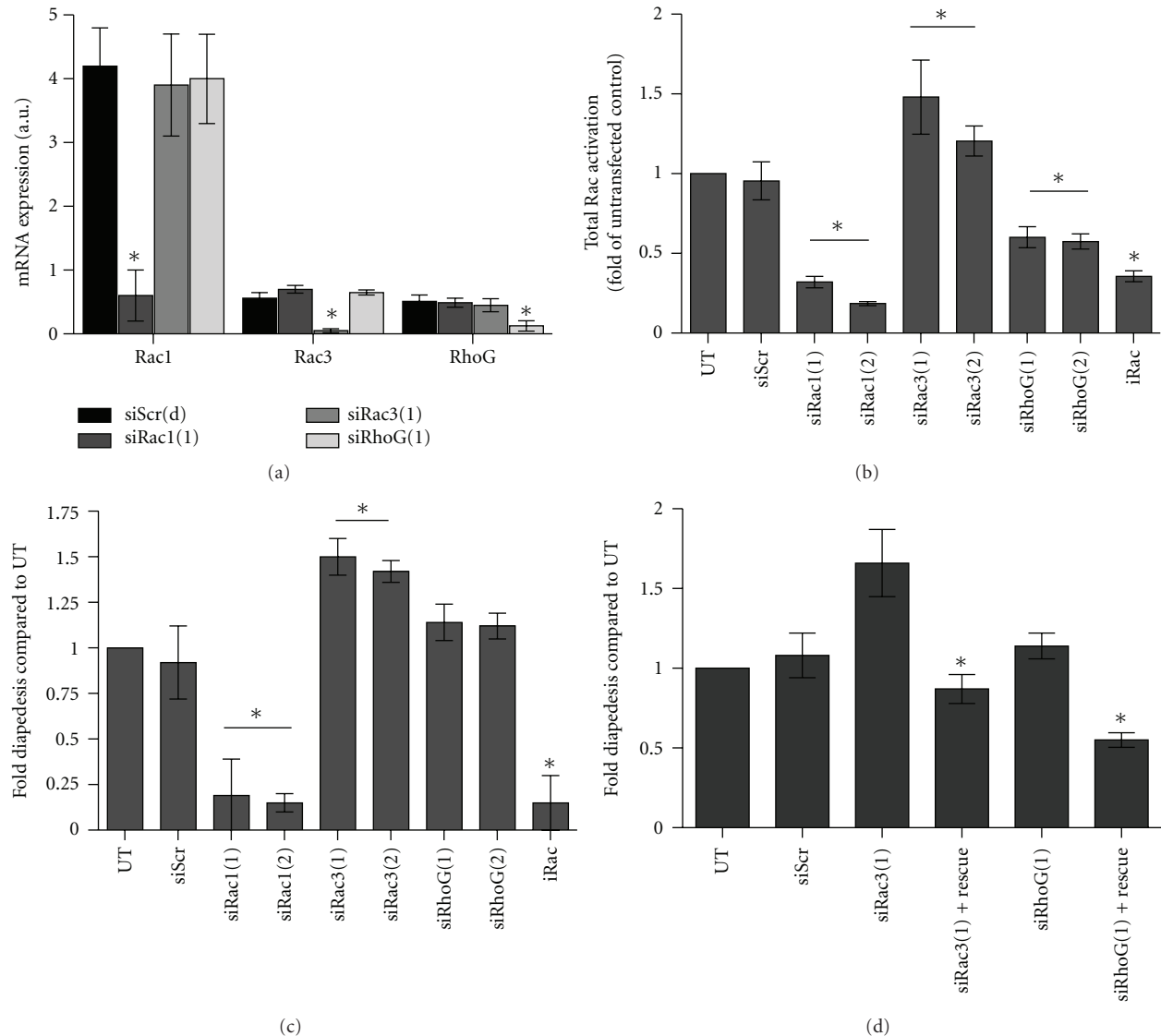
Figure 1: Effect of Rac depletion on total Rac activation and tumor cell diapedesis across a bone marrow endothelial cell monolayer. (a) Rac isoform expression and isoform-specific depletion in PC-3 cells. Individual siRNAs (1) and (2), specific for Rac1, Rac3, or RhoG, were compared. Messenger RNA was harvested and SYBR green-based qPCR performed using primers specified in Supplemental Table 1. Relative expression levels were normalized to GAPDH expression from the corresponding sample and expressed as arbitrary units (a.u.). (b) is the effect of the RacGEF inhibitor NSC23766 (iRac), siRNA specific for Rac1, Rac3, and RhoG, or scrambled control (siScr) on total Rac activation. Cells were treated with $100\,\mu$M NSC23766 for 1 h or $20\,\mu$M siRac1, siRac3, or siRhoG. Activation of total Rac was performed using GLISA. Cells treated with iRac or transfected with siRNA to Rac isoforms were compared with untransfected (UT) and representative siRNA-scrambled control (siScr). Each analysis was performed in triplicate with individual transfections. (c) BMECs were layered onto a Matrigel-coated filter and allowed to form a monolayer; 0.5 mL of a suspension of 3.75×10^5 PC-3 cells/mL were added to the BMECs and allowed to undergo diapedesis for 24 h. Treated and transfected cells were compared with untransfected or scrambled controls. (d) Introduction of an RNAi-insensitive Rac3 into siRac3-treated PC-3 cell. Shown in all four panels is the mean ± S.D. of at least triplicate analysis with significance being *$P < .001$. Noncapped lines above the bars represent that the siRNA group is significantly different from controls.

RhoG on tumor cell diapedesis. Supplemental Figure 2(b) demonstrates a similar trend for the C4-2 prostate cancer cells. Depletion of Rac1 led to a significant decrease in transendothelial cell migration. However, depletion of Rac3 or RhoG increased tumor cell diapedesis. Rescue experiments reversed the trends of the siRNAs in the C4-2 cells.

3.2. The Chemokine CCL2 Stimulates Diapedesis via RhoG GTPase. The chemokine CCL2 is produced by BMECs and stimulates Rac1-mediated tumor cell diapedesis [17, 32]. Since RhoG appears to have an effect on Rac activation, we next set out to determine if CCL2-stimulated diapedesis could be affected by depletion of RhoG. As shown in Figure 2(a), CCL2 treatment increased diapedesis 3-fold

(a)

(b)

(c)
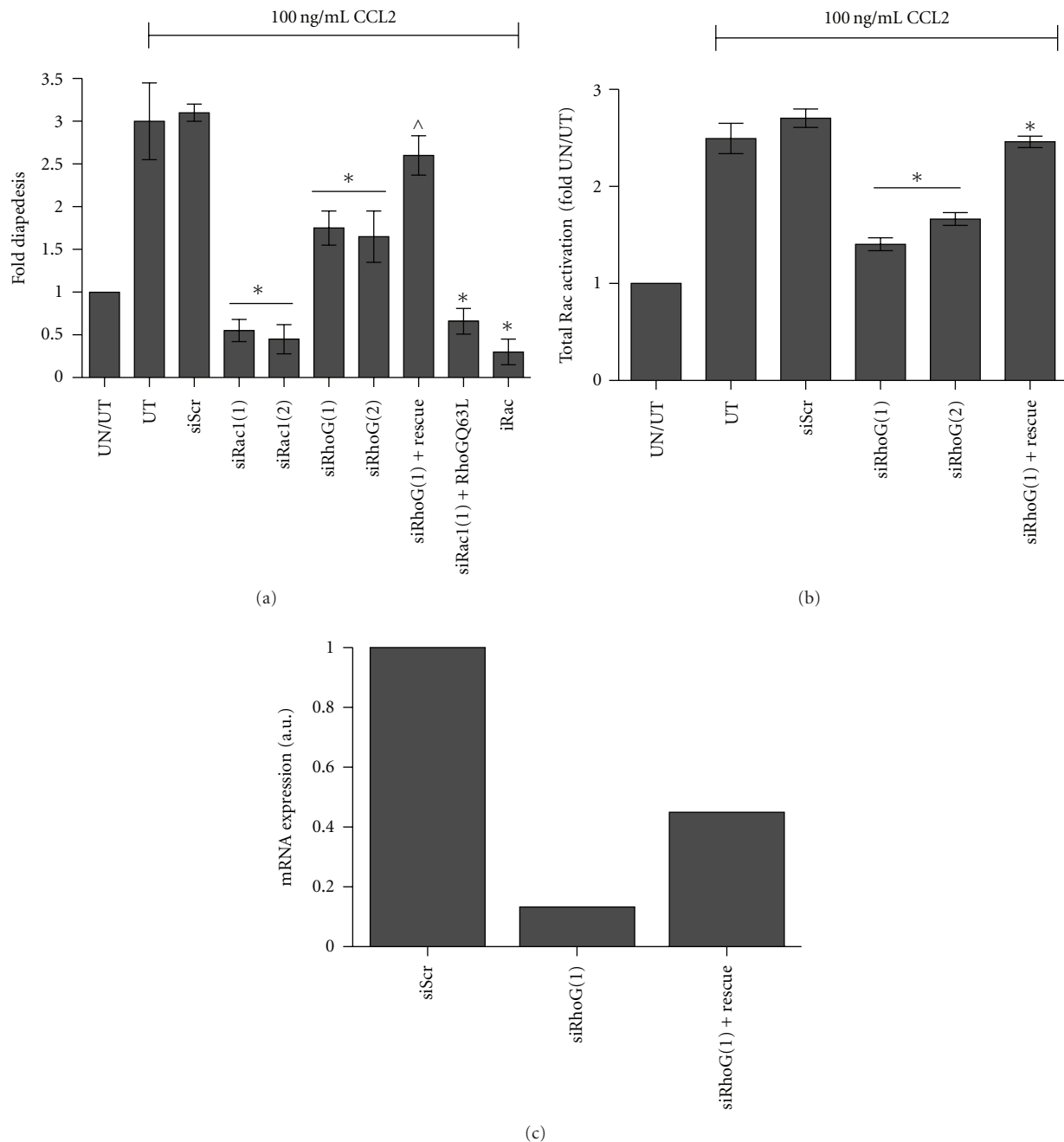
Figure 2: Effect of Rac depletion on tumor cell diapedesis across a BMEC monolayer. (a) PC-3 cells were treated with 100 ng/mL CCL2 in a diapedesis assay. Control untransfected (UT) and siRNA control (siScr) cells demonstrated increased diapedesis compared with untreated/untransfected (UN/UT) PC-3 cells. The ability of cells to undergo CCL2-stimulated diapedesis after depletion of Rac1, RhoG, or treatment with iRac was compared to UT and siScr. Rescue experiments of RhoG-depleted cells were performed by the introduction of a siRNA-insensitive RhoG. Rac1-depleted cells were rescued with the introduction of fast cycling RhoG (RhoGQ63L). (b) Depletion of RhoG led to a decrease of total Rac activation in PC-3 cells treated with 100 ng/mL CCL2. Rescue experiments were performed by introducing a siRNA-insensitive RhoG GTPase. Shown are means ± S.D. of at least triplicate analysis representing individual transfections, with significance being $P < .001$; (*) signifies a significant difference between siRNA-transfected cells and stimulated controls, while (^) signifies a significant difference between siRNA-transfected and -rescued cells.

across a BMEC layer in untransfected (UT) and siScr control cells compared to untreated/untransfected cells (UN/UT). Contrary to what we observed for unstimulated diapedesis in Figure 1(c), there was an approximate 45% decrease in PC-3

diapedesis across the endothelial cell layer when RhoG was depleted using siRNAs ($P < .001$). Similarly, direct depletion of Rac1 or treatment with the inhibitor NSC23766 led to a significant decrease in transendothelial cell migration.
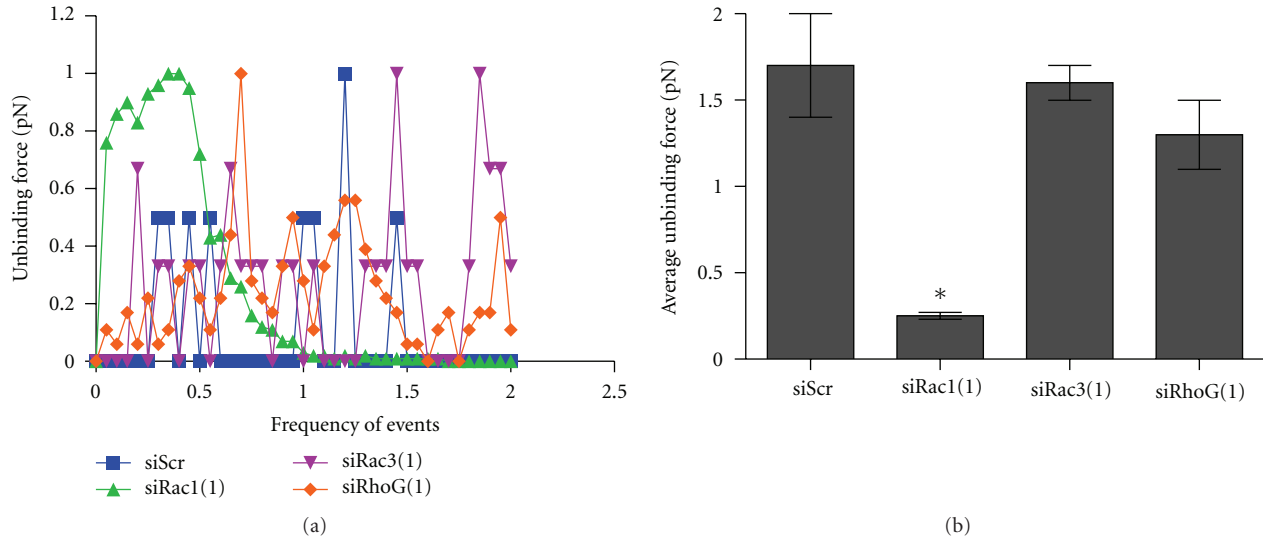
FIGURE 3: Interaction of prostate cancer cells with bone marrow endothelial cells BMECs was attached to the AFM tip and the unbinding forces of PCa cells measured. PC-3 cells were transfected with siRNAs specific for individual Rac isoforms. Shown are the results from one set of siRNAs. (a) Effect on the frequency of unbinding events and forces (pN) occurring between BMECs and PC-3 cells after depletion of each Rac isoform. (b) Average unbinding force occurring between BMECs and PC-3 cells. The average unbinding force is the physical force required to pull two adhered cells apart. Data are compiled from 3000 data points and are the mean ± S.D. with significance being *$P < .001$.

Introduction of a siRNA-resistant RhoG fully rescued CCL2-induced diapedesis in RhoG-depleted cells. However, introduction of a RhoGQ63L fast cycling mutant did not rescue the cells ability to cross an endothelial cell layer when Rac1 was depleted. Finally, Supplemental Figure 2(c) demonstrates that CCL-2-induced diapedesis is inhibited in C4-2 cells when RhoG is depleted. Again, introduction of a siRNA-resistant RhoG fully restores the cells ability to cross the BMEC layer.

Figure 2(b) demonstrates that CCL2-induced total Rac activation is decreased by ~40% when RhoG is depleted from the PC-3 cells, suggesting that CCL2 may activate Rac1 directly and also indirectly through RhoG GTPase. Concordant to what is observed in the diapedesis assay, introduction of a siRNA-resistant RhoG restores actives levels of total Rac similar to controls. Restoration of Rac activity and PCa diapedesis in the rescue experiments were not due to overexpression of nonphysiologic levels of ectopic RhoG. As shown in Figure 2(c), during rescue, mRNA levels of RhoG were increased 4-fold over the RhoG-depleted cells. These expression levels were still well under what is observed for the siScr control cells. Similar results were observed for the C4-2 cells and in the RNAi-insensitive Rac1 rescued cells. On average, an ~70% transfection efficiency was observed for each construct in both the PC-3 and C4-2 cells.

3.3. Rac1 GTPase Mediates the Interaction between PC-3 Cells and BMECs.

We previously demonstrated that downregulation of Rac1 does not significantly affect PC-3 cell binding to BMECs [18]. However, anecdotal evidence suggested that Rac1 depletion leads to decreased binding strength of the PC-3 cells to BMECs. To quantitate binding strength, we used atomic force microscopy (AFM) to measure the unbinding force of PC-3 cells bound to BMECs after Rac1, Rac3, or RhoG depletion. For the siScr control, siRac3- and siRhoG-treated PC-3 cells, a number of individual unbinding events occurred over time (Figure 3(a)) suggesting tight binding of multiple adhesion molecules is involved in cell-cell contact. In contrast, down-regulation of Rac1 led to a significant decrease in the number and frequency of unbinding events that occurred, suggesting fewer and weaker cell-cell contacts. Figure 3(b) shows that depletion of Rac1 led to a significant average 85% decrease in the unbinding force of the PCa cells to the bone marrow endothelial cells. Interestingly, downregulation of RhoG did not affect the ability of the PC-3 cells to bind to the BMECs, suggesting that RhoG activation of Rac1 is not involved in cell-cell binding.

In a system resembling initial contact during diapedesis, PC-3 cells were allowed to bind to a BMEC monolayer, and the dynamic elastic modulus (a.k.a. Young's modulus) was measured using AFM. Because of the pronounced effect of Rac1 depletion on PCa cell adhesion to BMECs seen in Figure 3, we compared siScr control and siRac1-transfected PC-3 cells. Figure 4(a) shows that the elasticity was essentially unchanged for the siRNA-scrambled control and siRac1 PC-3 cells alone. Compared to the unbound cells, the siRNA control PC-3 cells became more elastic (a decrease in the Young's modulus) when bound to BMECs suggesting that they begin to spread onto the endothelial cell monolayer. In contrast, the PC-3 cells transfected with siRNA to Rac1 were significantly less elastic (increase in the Young's modulus) than the unbound PC-3 cells when bound to BMECs suggesting that they remain in a rounded configuration as we previously reported [16]. Figure 4(b) compares the elasticity of the cells in the BMEC monolayer when engaged
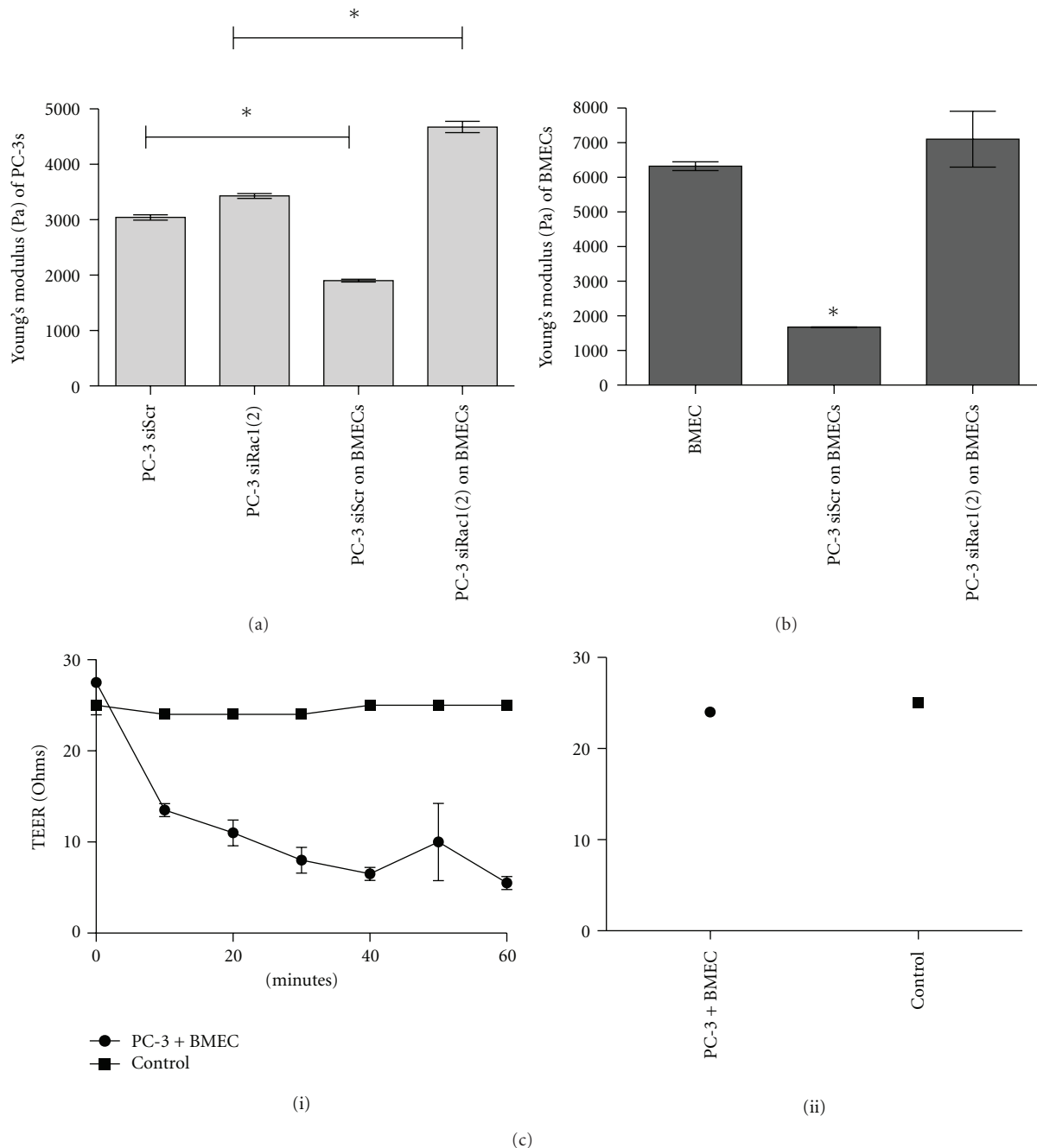
Figure 4: Bone marrow endothelial cells react to PCa cell binding. (a, b) are measurements of the dynamic elastic modulus or elasticity of PC-3 and BMECs in contact with one another. Elasticity is given as the Young's modulus and is a ratio of cell stress and strain and is measured in Pascals. BMECs were grown as a monolayer and control (siScr) or siRac1 expressing PC-3 cells were allowed to bind to the BMEC monolayer, and the elasticity of the PC-3 cells (a) and the BMECs (b) was measured by AFM. Data are the result of over 10,000 data points and represented as mean ± S.D. with significance being *$P < .001$. (c) are measurements of transendothelial electrical resistance (TEER). BMECs were grown on a monolayer, PC-3 cells were added to the monolayer, and the electrical resistance was measured every 10 min up to 1 h (i) and the final measurement at 24 h (ii).

by the PC-3 cells. BMECs had a significant 30% increase in elasticity when in contact with control PC-3 cells suggesting reorganization of the actin cytoskeleton. In contrast, the BMECs had no change in theirdynamic elastic modulus when bound to Rac1-depleted PC-3 cells.

In a variation of this experiment, we allowed individual BMECs to come into contact with a PC-3 cell monolayer (Supplemental Figure 3). Again, the elasticity of the PC-3 cells was essentially unchanged due to downregulation of Rac1. There was a significant and consistent 30% increase in

(a)

(b)
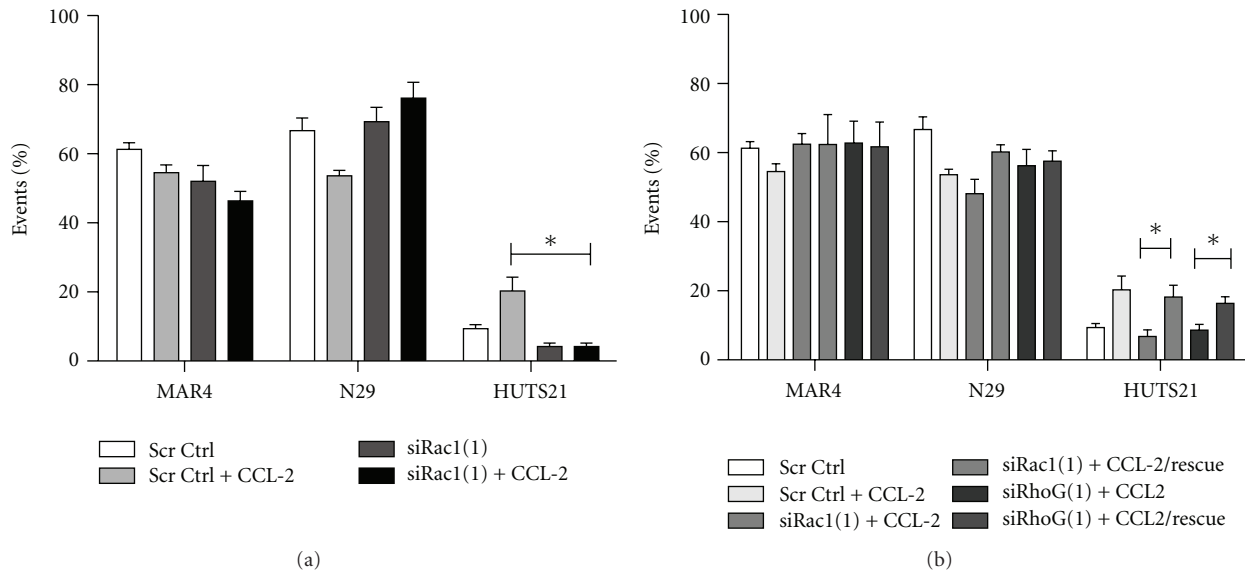
Figure 5: Effect of Rac1 GTPase on $\beta 1$ integrin activation. (a) Comparison of total, partially and fully activated $\beta 1$ integrinsin control and Rac1 depleted PC-3 cells as assessed by FACS analysis. Cells were transfected with either siRNA-scrambled control or Rac1 siRNA(2) and left unstimulated or treated with 100 ng/mL CCL2. FACS analysis was performed after incubating fixed cells with the antibodies MAR4 (for total $\beta 1$ integrin), N29 (for partially active $\beta 1$ integrin), and HUTS21 (for fully active $\beta 1$ integrin). (b) is a rescue experiment demonstrating that introduction of either an RNAi-insensitive Rac1 or RhoG GTPase leads to restoration of active $\beta 1$ integrin levels in CCL2-treated PC-3 cells. Shown are the results of triplicate experiments showing the percentages of 10,000 gated events with significance being $*P < .001$. Capped lines signify a comparison and significance between siRNA-depleted cells and cells rescued with siRNA-insensitive constructs.

elasticity of the BMECs when they came in contact with the control PC-3 cells. However, there was no change in elasticity of the BMECs when they came into contact with PC-3 cells that had depleted Rac1.

Figure 4(c)(i) demonstrates a marked change in transendothelial electrical resistance (TEER) across the BMEC layer. TEER is a measure of the integrity of tight junctions between cells. Decreased TEER is indicative of cellular retraction. Addition of PC-3 cells to a confluent BMEC layer led to a significant, time-dependent decrease in TEER. TEER levels were fully restored, in a time-dependent manner by 24 h (Figure 4(c)(ii)). Taken together these data suggest that the BMECs undergo cytoskeletal changes that influence their elasticity when they interact with PC-3 cells.

3.4. Active Rac1 GTPase Leads to Stimulation of $\beta 1$ Integrins. Active Rho GTPases are known to lead to expression and activation of integrins [36]. Two integrin heterodimers are associated with binding to VCAM-1 and ICAM-1 on endothelial cells, $\alpha 4\beta 1$ and $\alpha L\beta 2$, respectively. With this in mind, we set out to determine if Rac1 GTPase influenced the activation state of integrins leading to BMEC binding. Since $\beta 2$ integrins are not associated with prostate cancer and the role of the $\beta 1$ integrins is established in PCa/BMEC interactions [6, 8, 9], we focused on the expression and activation of the $\beta 1$ subunits. To determine this fluorescence-activated cell sorting (FACS), analysis was performed using a set of antibodies that recognize total and active levels of the $\beta 1$ subunit. The MAR4 antibody recognizes total $\beta 1$ integrin subunit regardless of activation state. The $\alpha 4\beta 1$

heterodimer can exist in 3 conformations, closed headpiece/bent (inactive), closed headpiece/extended (partially activated, recognized by the N29 antibody), and open headpiece/extended (fully active, recognized by the HUTS21 antibody). Figure 5(a) demonstrates that unstimulated PC-3 cells have similar levels of total and partially activated $\beta 1$ integrin as compared to Rac1-depleted PC-3 cells. When the cells were treated with CCL2, thus leading to increased Rac1 activation, there was no change in total and partially activated levels of $\beta 1$ integrin. However, significantly more fully activated $\beta 1$ integrin was detected in the control but not Rac1-depleted PC-3 cells. For simplicity, the results shown are from one siRNA; however, near identical results were obtained with alternate siRNAs.

Figure 5(b) demonstrates that the decrease in CCL2-stimulated $\beta 1$ integrin activity due to Rac1 depletion can be rescued by expression of an RNAi-insensitive Rac1 GTPase. Similarly, depletion of RhoG GTPase led to a significant decrease in CCL2-induced active $\beta 1$ integrin expression as compared to scrambled control. Expression of a siRNA-resistant RhoG led to a significant increase in $\beta 1$ integrin activation. In both cases, the RNAi-insensitive GTPases restored CCL2 activation of $\beta 1$ integrin to levels comparable to the control cells.

4. Discussion

The Rho GTPases comprise a subfamily of the Ras superfamily of monomeric GTP-binding proteins [21]. Like Ras, the Rho proteins transiently move from an inactive to active

to an inactive state via the GTPase cycle. This cycle is controlled by a number of regulatory proteins, which in turn regulate Rho signal transduction via effector proteins [37–41]. This coordinate regulation of the Rho proteins allows for cytoskeletal reorganization leading to changes in cell shape and motility [12, 42]. Overexpression and/or aberrant activation of individual Rho GTPases has been shown in a number of cancers and is thought to drive metastatic progression [15]. Although one Rho protein may be the predominant GTPase in a cancer, other GTPases must also become active to reorganize the actin cytoskeleton and drive migration.

RhoC GTPase is expressed in several cancers and promotes metastasis [43–52]. Previously, we demonstrated that RhoC GTPase expression and activation is required for PCa invasion [16, 18–20]. When RhoC expression or activation is downregulated, the PCa cells undergo Rac GTPase-mediated EMT [16, 18]. Decreased Rho expression or activity leads to increased expression and sustained activity of Rac1. Furthermore, Rac expression and activation was found to be required for tumor cell diapedesis across a human BMEC layer. We believe that together RhoC and Rac are needed to drive PCa extravasation from the vasculature into the bone marrow environment.

There are four members of the Rac branch of the Rho subfamily: Rac1, Rac2, Rac3, and RhoG. Rac1 and Rac3, but not Rac2, are shown to have increased expression in PCa, while expression of RhoG has not been examined [33]. In the current study, we set out to determine the individual roles of Rac1, Rac3, and RhoG in tumor cell diapedesis. Rac1 levels are significantly higher than either Rac3 or RhoG suggesting that it is the predominant Rac GTPase in these cells. The relative levels of Rac1 and Rac3 in PCa are similar to what has been shown in glioblastoma cells [26].

Rac3 GTPase has clearly been shown to be involved in adhesion of tumor and normal cells of neural origin [24, 26]. Normal and malignant prostate has a neuroendocrine component; therefore, the question arises if Rac3 expression plays a role in neuroendocrine differentiation of PCa [53–55]. We have clear AFM data that implicates Rac3 in binding of PCa cells to fibronectin and to a lesser extent, collagen I (unpublished data). Binding to laminin would be the next logical choice to examine. This aspect may also begin to explain the apposing effect that Rac3 has on Rac1 and transendothelial cell migration. We found that downregulation of Rac3 led to an increase in total Rac activity, independent of an increase in total Rac protein levels. A similar observation was made previously; downregulation of RhoC increased Rac1 activity [16, 18]. However, this was accompanied by an increase in total Rac1 protein.

Expression of RhoG is found ubiquitously throughout the body, but its expression in PCa has not been studied. We found that RhoG, although expressed in low levels, has an effect on total Rac activation. Inhibition of RhoG led to a significant decrease in Rac activation, but diapedesis was slightly increased. In contrast, CCL2-stimulation of PCa cells transfected with siRNA specific for RhoG significantly decreased diapedesis. Coexpression of a siRNA-resistant RhoG led to restoration of the cells ability to

cross the endothelial cell layer. Furthermore, depletion of RhoG led to a significant decrease in CCL2-stimulated Rac activation, suggesting that CCL2 activates Rac1. Expression of a fast cycling RhoG in Rac1-depleted cells did not rescue the cells ability to undergo diapedesis. This also suggests that Rac1-mediated diapedesis may be regulated through direct activation of Rac1 or indirectly via RhoG GTPase and the different effects that RhoG has on PCa diapedesisis intriguing. Without CCL2 stimulation, RhoG appears to act like Rac3 and limit diapedesis, even after decreasing total Rac activation. Upon CCL2 stimulation, RhoG appears to play a role in activating Rac1 thereby decreasing diapedesis. This may suggest a specific RhoG GEF(s) that are activated by CCL2. Also of interest is the fact that ectopic expression of an RNAi-insensitive RhoG led to a significant decrease in unstimulated diapedesis suggesting a balance of RhoG expression required for migration.

Our results measuring BMEC stiffness using AFM showed specific differences that were consistent over a large array of experimental attempts suggesting a specific biological interaction. The PC-3/BMEC interaction is of particular interest; PC-3 cells, whether adhered to substrate or attached to a BMEC, maintain a constant measured elastic modulus. The BMECs, however, when in contact with a PCa cell consistently, undergo a 30% decrease in stiffness. This apparent conferred decrease in stiffness points to a change in the internal cytoskeletal architecture of the BMEC. Depletion of Rac1 in the PC-3 cells led to a significant decrease in the strength of binding to the BMECs. The elasticity of the BMEC cell was not decreased when bound to a Rac1-depleted PC-3 cell indicating a Rac1-mediated interaction between the two cells. This interaction may be due, at least in part, to binding mediated by $\beta 1$ integrins. We demonstrate that unstimulated PC-3 cells have partially activated $\beta 1$ integrins that become fully activated upon CCL2 stimulation and activation of Rac1. Clearly, $\beta 1$ integrins are required for binding of PCa cells to extracellular matrix [7, 19, 20, 56, 57]. Studies in the literature suggest a role for $\beta 1$ integrins in binding PCa to BMECs [6, 7, 58, 59]. One report suggests that the use of a $\beta 1$ integrin-blocking antibody did not affect PC-3 cell binding to the human bone marrow endothelial cell line HBME-1 but was responsible for mediating PCa interactions with fibronectin [7]. However, other studies show a role for $\beta 1$ integrins in binding to other bone marrow endothelial cells [58, 59]. Rho GTPases such as Rac1 are implicated in bidirectional signaling with integrins activating Rho proteins and the active Rho proteins promoting integrin dimer activation increasing binding strength [36, 60, 61].

These mechanisms are similar to leukocyte diapedesis, where, after initial binding, the interaction between the leukocyte and endothelial cell increases leading to dynamic cytoskeletal changes and endothelial cell retraction [62, 63]. Although this has been suggested for PCa diapedesis, this is the first time this has been shown experimentally. A complete understanding of how these different Rac proteins are activated and how they contribute to tumor cell diapedesis may have profound implications for any strategies targeting the extravasation process.

Conflict of Interests

The authors have no conflict of interests to disclose.

Abbreviations

PCa: Prostate cancer
Rho: Ras homology
dnRho: Dominant negative Rho
shRNA/siRNA: Small hairpin RNA/small inhibitor RNA
FBS: Fetal bovine serum
BMEC: Bone marrow endothelial cell
ECM: Extracellular matrix
AFM: Atomic force microscopy.

Acknowledgments

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Extended Antiandrogens Evade Prostate Cancer Resistance in Vitro and in Vivo.

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Abstract

Antiandrogens remain the standard of care for advanced prostate cancer. Despite an initial favorable response, prostate cancer often acquires resistance to antiandrogen treatment, which can result from a number of mechanisms including gain of function mutations to the androgen receptor (AR), AR over expression, de novo synthesis of androgens and alterations in paracrine communication within the tumor microenvironment . Many antiandrogen-resistant cancers ~~may still be~~ remain responsive to antiandrogens of a different structural class. We developed new antiandrogens having extended structures designed to disrupt more effectively ~~disrupt~~ cofactor binding. These compounds, that were effective in vitro against wild-type and mutant ARs, were evaluated for their ability to prevent formation of resistant clones using LNCaP and CWR22-Rv1 cells in long term in vitro selection assays ~~using LNCaP and CWR22-Rv1 cells~~ and was correlated with their activity in coactivator association, AR down-regulation and AR nuclear localization assays. The ability of compounds to prevent resistant colony formation correlated with their ability to reduce nuclear localization of AR, which in turn correlated with increasing antiandrogen size. Further, ~~T~~the compound PLM6 was ~~further~~ found to induce apoptosis within the prostate epith~~ei~~lium of balb/c as well as tgfbr2-Col-KO mice, the latter of which are a model for "outlaw" pathway castration resistance. Collectively, these studies suggest that appropriately designed antiandrogens may be more effective in combating mechanisms associated with current antiandrogen failure.

Introduction

Prostate cancer (PCa) remains the second leading cause of cancer death in men.[1] Advanced ~~prostate cancer~~ PCa is treated commonly ~~treated~~ with antiandrogen strategies~~s~~ ~~which~~ that can inhibit the biosynthesis of dihydrotestosterone (DHT) or are antagonists of the androgen receptor (AR). However, current antiandrogens, though initially effective, eventually become ineffective as cancers progress to castrate resistant state.[2] A number of mechanisms cause antiandrogen resistance including: AR over-expression, which is often associated with gene amplification,[3] acquisition of mutant ARs that are activated by antiandrogens or other ligands,[4] constitutively active forms of AR,[5] local biosynthesis synthesis of DHT and hormone independent activation of AR signaling pathways by cytokines/receptor tyrosine kinase signaling cross talk.[2c, 6]

Despite the characterization of these cancers as being castrate resistant as they progress under conditions of reduced circulating androgens, several lines of evidence suggest that androgen receptor signaling may still be operative in hormone independent disease.[2b] For example, 27-40% of patients who have become resistant to an initial antiandrogen treatment respond favorably to 2nd line antiandrogen treatment.[7] Similarly 53% of patients whose cancers progress under antiandrogen therapy show improved symptoms, though temporary, to discontinuation of antiandrogen treatment.[2c, 8] These studies support the notion that antiandrogens can still play a role in the treatment hormone refractory/castration resistant disease and that antiandrogen resistance is, in part, defined by and limited by the current repertoire of available antiandrogens. Herein, we describe a new class of antiandrogens developed to resemble more closely resemble the structures of effective antagonists of related nuclear/steroid hormone receptors that use long polar extensions to more fully disrupt cofactor interactions and their preliminary evaluation in vitro and in vivo.

Androgen receptor antagonists can inhibit the DNA binding domain or the C-terminal ligand binding domain. Common antiandrogens, e.g. flutamide, nilutamide, bicalutamide (Bic), and cyproterone acetate, competitively bind the ligand binding domain. However, these antiandrogens fall outside the structural paradigm of nuclear and steroid hormone receptor antagonists that follow the "extension hypothesis" wherein antagonists have more extended structures than agonists and protrude or extend out of the ligand binding pocket to disrupt cofactor interfaces.[9] These compounds are potent and efficacious AR antagonist in vitro and work well against naive PCas but routinely are rendered clinically ineffective by a variety of cellular and biochemical mechanisms.

A few studies recently have recently reported antiandrogens that are indeed somewhat larger than bicalutamide that may provide advantages against the development of antiandrogen resistant disease.[8, 10] As part of our program to evaluate new antiandrogens directed towards evading common mutations associated with bicalutamide resistance, we constructed a new series of antiandrogens PLM1, PLM2 and PLM6, that contained expanded arylsulfone structures relative to bicalutamide.[10a] All three compounds were found to be low (EC50 <20 µM) micro molar potent panantagonists of both wild-type and mutant AR:, AR (W741L), AR (W741C) and AR (T877A), known to be associated with antiandrogen withdrawal (Figure 1c). Whereas these studies suggest that these analogs may have an advantage in AR mutants associated with flutamide and bicalutamide resistance, they do not preclude the possibility that other AR mutants

or ~~other~~additional cellular changes that may emerge under selective pressure might render them ineffective. Rather than presuming a specific mechanistic cause for antiandrogen failure and screening our compounds in mechanism-specific biochemical assays, we initiated a program to examine the susceptibility of antiandrogens to acquired resistance using long-term in vitro selection assays as part of a compound development program.[11] These have inspired a second generation of extended antiandrogens that are showing promising activity in vitro and in vivo.

Results

Castrate resistant growth.

Resistance to AR antagonists is common. LNCaP cells were cultured with 10 µM PLM1, PLM6 and bicalutamide long term (10 weeks). Under these conditions we found that the behaviors of bicalutamide, and the pan-antagonists PLM1 and PLM6 to bewere markedly different. All three antagonists initially caused an overall decrease in cell proliferation that appeared identical over the first 4 weeks of cultureing. However, with further culturing, resistant colonies emerged from the Bic and PLM1 treated cells. In fact, PLM1 treated cells overgrew the plates at 12 weeks. In contrast, cells treated with PLM6 formed only one resistant focus of cells in i out of three independent experiments whichthat were otherwise terminated after week 10, when no other viable cells could be detected (Figure 2a). At lower doses, cells treated with 1µM PLM6 did not select for resistant clones and thus compared favorably to Bic and PLM1 (SI Figure 1). The ability of PLM6 to reduce dramatically reduce the formation of resistant coloniesy formation did not appear to be due to indiscriminate cellular toxicity as PLM6 shows no significant toxicity over vehicle or bicalutamide in CV1 cells or AR negative PC3 cells at the concentrations used for selection. Nor was this property simply a reflection of greater potency assince PLM6 is less potent than PLM1. Growth inhibition assays also indicate that the GI_{75} of PLM6 was 5-times lower than that of Bic (SI Figure 1). One obvious structural difference was that between the PLM1 and PLM6 is that PLM1 is only marginally larger than bicalutamide. PLM6, however, was structurally the largest and the most effective at evading resistance under in vitro selection. We therefore speculated that thePLM6's superior performance of PLM6 in vitro might be attributed to its larger size perhaps by its ability to better disrupt the active conformation of the AR ligand binding domain and its association with cofactors. This would suggest that making larger antiandrogens might be more effective at evading acquisition of resistance in vitro. We therefore explored modification of PLM1, PLM6 and PLM2 with long polar extensions similar to those found in antagonists of other steroid receptors.

We found that dimethylaminopropoxy substitution of the 2' and 3' positions of PLM6 created new analogs, PAN52 and PAN62, that were also were panantagonists of wild-type AR and AR mutants. The long polar extensions at these positions could be done with little loss in potency and in some cases actually may have actually improved potency of the ligand relative to the parent compound PLM6. These studies demonstrate that androgen receptor antagonists having long polar extensions can retain panantagonist activity while maintaining potencies similar to Bic

(IC$_{50}$ 1.0 ± 0.08 µM). These data prompted us to evaluate further our compounds using in vitro selection assays.

We selected PAN52 as a prototypical example of an extended antiandrogen based on its cellular potencies and efficacies. We compared PLM1, PLM6 and PAN52 to bicalutamide and vehicle controls in AR expressing LNCaP and CWR22-RV1 cells in soft agar growth assays. By using agar imbedded cells, these studies eliminated potential artifacts associated with cell detachment that could be observed at times in prior selection studies using adherent cultures. LNCaP cells were selected as a prototypical example of an antiandrogen resistant cell line that was derived from a flutamide resistant tumor.[12] CWR22-Rv1 are a castrate resistant cell line that has not been exposed previously to antiandrogens.21 LNCaP cells treated for three weeks with 1 µM and 10µM PLM1, PLM6, Pan52 and bicalutamide all suppressed colony formation relative to vehicle control and essentially were indistinguishable from each other (SI-#). However, with continued culturing, cells treated with 1 µM PLM1, PLM6 and bicalutamide but not PAN52 began to form distinct foci by week 6 (Figure S#). At 10 µM all antiandrogens repressed LNCaP focus formation. Unlike our prior studies with LNCaP cells in adherent culture, attachment independent growth of LNCaP cells failed to differentiate between bicalutamide and PLM6 at the concentrations tested; however, PAN52 prevented formation of resistant foci even with extended culturing.

For CWR22-Rv1 cells treated with 10 µM antagonist, all antiandrogens suppressed soft agar colony formation relative to vehicle control and essentially were indistinguishable from each other after 3 weeks of culture. However, with prolonged culturing, PLM1 and Bic, but not PLM6 or PAN52, developed foci that were visible clearly after 6 weeks of culturing (Figure 2d). Bic and PLM1 treated and resistant CWR22-Rv1 foci were isolated and cultured after 16 weeks in soft agar remained resistant to bicalutamide and PLM1. No live CWR22-Rv1 cells could be detected in PAN52 treated cells after 16 weeks; however, a few MTT positive cells were detected in the PLM6 group (SI). Toxicity studies with PAN52 in AR-negative PC3 cells indicated no significant differences between PLM6, PLM1 and bicalutamide or compared to vehicle control at the concentrations used for selection. Some toxicity of PAN52 and PLM6 was observed in PC3 cells at concentrations ≥50 µM (Figure 2c). These findings combined with the emergence of resistant clones to conventional antiandrogens only after 4 weeks support the notion that PLM6 and PAN52 have a unique ability to suppress the emergence of resistant cells that was distinct from generic compound toxicity.

Using in vitro selection was effective at differentiating between analogs that evaded selection of resistant subclonal populations in vitro; however, it did not establish if these are properties unique to the LNCaP and CWR22-Rv1 cell lines in vitro or if resistance correlates with specific mechanisms associated with antiandrogen failure. We therefore evaluated each of these compounds using in vitro assays corresponding to potential mechanisms of resistance. Further, the specific efficacy to bicalutamide resistance was determined in a LNCaP colony cloned following 16 weeks of bicalutamide culture. As with CWR22-Rv1 cells, the bicalutamide-resistant LNCaP cells proliferated in the presence of bicalutamide and PLM1, but not PLM6 and PAN52 (SI Figure 3). Together these studies suggested castrate dependentsensitive and resistant prostate cancer cells can develop AR dependent resistance to bicalutamide and PLM1, but PLM 6 and PAN52 may have a unique advantage in evading resistant clones derived during long term culturing.

Cofactor recruitment:
Residual or altered cofactor recruitment has been proposed as a predictive measure of antiandrogen function.13, 22, 23 Cofactor recruitment was evaluated using TR-FRET association of cofactor derived peptides (Lanthascreen, Invitrogen). Using this assay, thes AR associates with the cofactor peptide D11 in the presence of DHT and some AR antagonists. Similar to previous reports, we also found that bicalutamide induced association of the D11 coactivator peptide at higher concentrations, although to a lesser degree than DHT.[8] 13 By contrast, PLM1, PLM6 and PAN52 showed no significant cofactor recruitment at the concentrations tested, suggesting that these larger antiandrogens may be more effective at blocking or reducing cofactor recruitment (Figure 2e).

PLM1, PLM6 and PAN52 down regulate AR
Downregulation of AR has been proposed as a possible new mode of action for antiandrogens that may help avoid antiandrogen resistanance, which is associated most often associated with AR overexpression.[3] A few reports have described compounds that affect downregulation of cellular AR levels in vitro.[13] Therefore we evaluated the ability of our analogs to down regulate AR expression in LNCaP cells and the castrate resistant CWR22-RV1 cells. LNCaP and CWR22-Rv1 cells treated for 22 hours with 10 nM DHT or 100 µM antiandrogen were lysed and analyzed by western blot for AR protein expression (Figure 3a and 3b). Treatment of LNCaP cells with Bic and DHT did not significantly alter AR protein levels significantly; however, PLM1, PLM6 and PAN52 induced strong downregulation of AR (Figure 3a). The AR down regulation

response is dose-dependent with low concentrations of antiandrogen (10 µM) showing little or potentially stabilizing effect on AR levels but increasing concentrations of antiandrogen ~~higher doses~~ decreas~~ed~~ing the levels of AR ~~with increasing concentrations of antiandrogen~~ (Figure 3c and supporting Information). In the presence of a protein translation inhibitor, cycloheximide (CHX), PLM6 was found to reduce ~~further reduce~~ AR downregulation further over CHX alone demonstrating that AR down-regulation by PLM6 is independent, at least partially, of protein synthesis. CWR22Rv1 cells contain a truncated from of the AR ~~which~~that lacks a~~n~~ C-terminal ligand binding domain (ARΔLBD). ~~which~~This truncated isoform also was ~~also~~ found to be downregulated by treatment with PLM1, PLM6 and PAN52 but not bicalutamide. These studies demonstrate that AR down regulation was a common feature of our second generation antiandrogens but down-regulation assays could not differentiate between compounds that readily succumbed to resistance in vitro (PLM1) and those that were able to suppress or evade resistance.

Nuclear localization:

Antiandrogens that block nuclear localization also have ~~also~~ been proposed as an alternative mechanism to reduce the potential for antiandrogen resistance.[8, 14] Using an AR-GFP fusion we examined ligand-induced cellular localization in transiently transfected HEK293t cells grown in hormone depleted media. Prior to treatment, AR-GFP is diffuse and predominantly cytosolic, however, 2 hours post treatment with DHT or Bic causes strong nuclear localization of AR-GFP (Figures 4). PLM1 has an attenuated nuclear localization response with cytoplasmic AR still clearly visible. For PAN52, AR-GFP remains diffuse in the cytoplasm and in the nucleus in some cases having nuclei hardly discernible from the cytoplasm.

To ~~further~~ evaluate ligand dependent nuclear localization of endogenous AR further, we evaluated nuclear versus cytosolic fractions of AR in LNCaP and CWR22-Rv1 cells grown in hormone depleted media. DHT treated cells showed a marked increase in nuclear AR; however, in contrast to studies performed by imaging AR-GFP, 10 µM bicalutamide did not show increased nuclear localization relative to vehicle controls. Similar differences between nuclear localization by Bic when assayed by GFP labeled AR and by cellular fractionation ~~have~~were observed by others.[15] In LNCaP cells, PLM1 was found to stimulate nuclear localization although to a lesser exten~~td~~ than DHT. PLM6 and Pan52 did not stimulate nuclear localization in LNCaP cells and had similar levels of nuclear AR as compared to vehicle (Figure 4g). The results were similar in CWR22-Rv1 cells where~~;~~ PLM1 stimulated strong nuclear localization;

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however, PLM6 and PAN52 appeared to reduce nuclear localization to levels below vehicle and Bic (Figure 4h). These studies demonstrate that PLM6 and PAN52, and to a lesser extent PLM1, cause reduceddecrease levels of AR nuclear localization compared to Bic.

Encouraged by our findings in vitro, we further explored the actions of PLM6 and Pan52 in vivo. Initial studies using Male C57Bl/6Balb/c mice treated with PAN52 or vehicle (ethanol) injected IP as 9:1 Cremophor: antagonist (resuspended in ethanol) at 25 mg/kg/day in intact (not castrated) mice for 3 or 5 days. Prostates were removed from 3 day and 5 day treatment groups, weighed, then fixed, sectioned and stained by H&E and TUNEL (Figure 5). PLM6 decreased prostate wet weights significantly by day 5 of treatment (Figure 5c) . PAN52 and PLM6 showed an increase in apoptosis as compared to control at both day 3 and day 5 (Figure 9, TUNEL). Upon quantification of TUNEL stained nuclei, levels of apoptosis were comparable between PAN52, PLM6, castrated controls and bicalutamide. Kidneys and livers were collected for histological analysis. No morphological abnormalities associated with hepatotoxicity or nephrotoxicity were observed at either time interval for PLM6 or PAN52. PLM6 showed no sign of renal toxicity while PAN52 showed some indication of multifocal cortical renal tubular vacuolation. The clinical implications of this finding are unclear (supporting information). Taken together these results suggest that PLM6 and PAN52 were well tolerated in vivo ;and were bioavailable to the prostate; and, induced to affect prostate regression through apoptosis similar to that observed for castration. [DONT WE HAVE 3Week studies too? WHAT IS THE FINAL WORD ON HISTOLOGY?]. ...
These studies demonstrate that PLM6 and PAN52 are well tolerated and are effective as antiandrogens in regressing the prostate in vivo.

We selected PLM6 for further evaluation in models for androgen independentcastrate resistant PCa models in vivo. First, we evaluated the ability for PLM6 to affect castrate resistant C4-2B xenografts in #### mice. NEIL NEED DETAILS> Mouse type, dosing, time, how measured, N for here and SI. The C4-2bB are a prototypical castrate resistant prostatePCa cell line derived from a spontaneous bone metastasies of the castrate resistant LNCaP derivative, C4-2-xenografts. Mice treated # mg/kg/day for 3 weeks reduced C4-2bB xenograft mass by 49% (P=0.0005) relative to vehicle. [does anyone have a reference or data to Bic response to C4-2b?]
The tumor microenvironment also plays an important role in prostate cancer development and progression and cannot be fully reproduced in vivo. The stromal specific knockout of the TGFβ

receptor II is sufficient to cause epithelial prostatic neoplasia and castrate resistancet offering a unique opportunity to evaluate PLM6 in this "outlaw pathway" resistance model.[16] We evaluated PLM6 in tgfbrColTKO mice compared to vehicle and bicalutamide controls. Mice were treated daily IP with Bicalutamide or PLM6 25#mg/kgd/day and bicalutamide for # days. *Explain assay details results and statistical significance etc.*   TUNEL analysis of isolated prostates revealed that Bic had no effect on apoptotic response over vehicle in the prostate similar to results reported for castration control. By contrast PLM6 induced a 2.8-fold increase in apoptotic response in the prostate indicating that new antiandrogens may offer distinct advantages over current antiandrogens such as Bic in castrate resistance models associated with cytokine signaling from the prostate microenvironment.

Molecular modeling

The results of cellular selections and biochemical studies suggest that appropriate expansion of the bicalutamide core can effectively access a new manifold of antiandrogen activity that is present in PLM6 and PAN52 but not in Bic and PLM1. Structural comparisons of each ligand suggest that the ability of antiandrogens to evade resistance in vitro correlates with molecular size (Figure 1b). PLM6 and PAN52 are considerably larger in size than current antiandrogens

currently or previously favored in clinical use including flutamide, nilutamide, cyproterone acetate and bicalutamide. Compounds smaller than PLM6 readily acquired resistance under clonal selection whereas PLM6 and PAN52 show an ability to evade formation of resistant subpopulations under in vitro selection. In order to obtain a better understanding of the binding mode of our analogs compared to other 2nd generation antiandrogens, we used molecular modeling to dock our analogs into constrained site models of AR(wt) and AR(W741L), an AR mutant towards which bicalutamide is an agonist. In the absence of a structure of the antagonist conformation of AR, modeling studies were performed with H12 deleted as crude but unbiased approximation of the antagonist conformation of the receptor.[10a] Whereas the structure of bicalutamide in AR(W741L) appears to stabilize the agonist conformation of helix-12, the docked structure of PAN52 is sterically incompatible with the agonist-bound conformation of helix-12.

By contrast PLM1 and the antagonists MDV3100 could be docked into the agonist conformation of intact AR(wt) and suggests that that MDV3100, though more potent, binds with a similar shape and size to PLM1 (Figure 1c).

Discussion

When placed under selective pressure prostate cancer can use several molecular mechanisms to circumvent low levels of circulating androgens and or the presence of antiandrogens. These include acquisition of AR mutations for which antiandrogens that are otherwise antagonists of AR(wt) act as agonists of the mutant, AR over-expression, which is sufficient to cause many antagonists to act as agonists and, androgen independent activation of AR through cytokine/ chemokine cross-talk often termed "outlaw pathway" resistance.[2a, 17] Additionally, local biosynthesis of DHT within the prostate and truncated forms of AR that lack a C-terminal ligand binding domain but remain constitutively active also have been identified as mechanisms for androgen independence or antiandrogen resistance.[5a, 18] Until recently, the failure of current clinical antiandrogens has been used to indict the entire class of AR antagonists; however, increasing evidence suggests that new antiandrogen designs may still play a role in treating castrate- and antiandrogen-resistant disease.

Our prior studies focused on how antiandrogens such as bicalutamide could be redesigned on the basis of structure to retain antagonist function with bicalutamide resistant mutants AR(W741L) and AR(W741C).[10a] We and others have predicted that mutations such as Trp741→Leu create additional space within the binding pocket such that the aryl sulphone no longer impinges upon and destabilizes the agonist conformation of helix-12 critical for cofactor binding.[4, 10a] While it may not be possible to design a ligand that is effective against all possible combinations of mutations that could arise under selection, antiandrogens with large extensions protruding into the space occupied by the agonist conformation of helix-12 should be less likely to serve as agonists towards ARs with mutations that result in enlargement of the hormone-binding pocket. Antiandrogens with long polar extensions protruding directly in to the coactivator binding space may be particularly effective in disrupting interactions of the hydrophobic coactivator interface.

The design of AR antagonists with long polar extensions similar to those found in efficient antagonsits of other steroid hormone receptor family members has been challenging as antagonist bound structures of AR have been resistant to crystalization.[19] The AR's long C-terminal (F-) domain that appears to constrain the conformation of helix-12 along with intramolecular N-to-C terminal interactions makes it difficult to use structure based approaches to design AR antagonists.[10d, 20] Miller et al. recently reported that that an analog of PLM1 co-crystalized with an AR mutant in its agonist conformation in a manner we predicted previously

for PLM1 through modeling.[21] Therefore acquired resistance to PLM1 in vitro may be consistent with the ability of AR mutants to still access their active conformation when bound to PLM1. Until recently there has been a limited number of reported AR ligands with structures that could be reasonably assumed to protrude out of the ligand binding pocket. Examples include cyanonilutamide conjugates,[10b, 13d] the PR antagonist mifipristone,[22] chalcones and the natural product Lupeol[REF].17, 44 We have identified several extended antiandrogens based on the well established arylsulfone scaffold that acts as panantagonists of AR and AR mutants in vitro. Although many antiandrogens are affective as AR antagonists in vitro or against naïve prostate cells in vivo, most current antiandrogens succumb to resistance when placed under the selective pressure in vivo. We applied in vitro selection assays early in the discovery process to evaluate the potential for antiandrogens to be thwarted by accessible resistance mechansims. In vitro selections have been explored by others but as yet have not been fully validated as a method to evaluate ligand suseceptiability to antiandrogen resistance prior to in vivo testing. [hara] Whereas in vitro culture cannot replicate the complex multicellular events associated with cancer progression in vivo, these assays may provide a low cost initial screen for a compound's propensity to succumb to resistance early in the discovery process. We performed in vitro selections using two different cellular backgrounds. Currently, we have no direct evidence for the formation of de novo mutations under our culturing conditions and therefore presume that the resistant clones emerge from lurker cell populations of the parent culture. LNCaP cells, which were derived from a flutamide resistant cancer, and CWR22-Rv1 cells, a castrate resistant PCa that has not been exposed previously to antiandrogens, provide distinct antiandrogen resistant and androgen independent cell backgrounds. Both cell lines over express the AR and LNCaP cells are known to harbor the AR(T877A) mutant associated with flutamide withdrawal syndrome wherein flutamide (but not bicalutamide) serves as an agonist.[ref]

Treatment of adherent LNCaP cultures demonstrated a clear advantage of PLM6 over PLM1 and bicalutamide, even when 1 µM PLM6 is compared to 10 µM bicalutamide and PLM1 (SI). This is a notable finding as it clearly demonstrates that the superior performance of PLM6 (IC50 = 8.5 µM) relative to Bic (EC50 = 1.0 µM) is not due to differences of inherent potency or efficacy. In attachment independent growth assays using CWR22-Rv1 cells, both PAN52 and PLM6 provided a clear advantage in blocking development of resistant foci as compared to Bic or PLM1. Significantly, these differences are not apparent during more typical short-term (<3

week) experiments wherein PLM1, PLM6, PAN52 repress growth relative to vehicle control similar to Bic. Only under under prolonged culturing, 6-8 weeks, do cellular clones emerge under selection. These studies suggest that PLM6 and PAN52 have a unique ability to evade selection of resistant clones in vitro under castrate resistant and antiandrogen-resistant cell lines. The ability for these compounds to evade formation of resistant clones parallels their molecular size; Bic < PLM1 < PLM6 < Pan52. We see no evidence that these differences are the result of generic toxicity as the compounds do not show appreciable toxicity at the concentrations tested and PLM6 and PAN52 havehas been tested in vivo at 100 mg/kg/day for three weeks without toxicity or discernible side effects (data not shown).

Reduced co-activator association and AR-down regulation have been suggested as possible mechanisms to reduce antiandrogen resistance.[ref] As has been shown by others previously, at higher concentrations, Bic bound AR can associate coactivator peptides in vitro by TRF. Under the same conditions, our analogs PLM1, PLM6 and PAN 52 did not show significant cofactor association by in vitro TRF assay. Significantly, D11 cofactor peptide association did not distinguish between PLM1, which readily succumbs to resistance in vitro, versus PLM6 and PAN52, which do not, thereby suggesting that D11 peptide recruitment may not be a reliable predictor of the ability of compounds to evade resistance in vitro.

Ligand-induced AR down-regulation also has also been suggested as a possible mechanism to circumvent resistance associated with AR over-expression, which is commonly associated with failure of classic antiandrogens. PLM1, PLM6 and PAN52, but not Bic, significantly down regulate AR protein levels in both CWR22 and LNCaP cells; however, similar to other reported AR down-regulators, strong down-regulation was observed at higher ligand concentrations than is necessary to suppress AR transcriptional activity. We again found AR down-regulation failed to distinguish between ligands that succumb to resistance (Bic and PLM1) and those that do not (PLM6 and PAN52).

Truncated forms of AR that lack a ligand-binding domain but retain constitutive activity also have also been implicated as another mechanism leading to antiandrogen failure and have prompted some to develop antagonists that target the N-terminal DNA binding domain of AR.[ref] PAN52 and PLM6 were found to be effective in suppressing growth and avoiding emergence of resistantt colonies in attachment independent CWR22Rv1, which expresses an AR isoform lacking a ligand binding domain (ARΔLBD).   Our analogs PLM1, PLM6 and PAN52 were found

to down-regulate levels of both full length and ARΔLBD consistent with the notion that the ARΔLBD in CWR22Rv1 result from proteolysis of a mutant AR.49. These studies also indicate that significant amounts of truncated AR, ARΔLBD, are present at the concentrations of PLM6 and PAN52 used to suppress emergence of CWR22-rv1 clones indicating that PLM6 and PAN52 can be effective with cell lines expressing truncated forms of AR. This is consistent with observations by Sawyers et al who suggested that transcriptional activity of ARΔLBD is dependent on the presence of the full length AR.9

Blocking or limiting the nuclear localization of the AR has been proposed as an alternative mechanism to shut down transcription by AR as increased nuclear localization has been associated with antiandrogen resistance.33 We evaluated the relative amounts of nuclear AR compared to vehicle control by nuclear localization by nuclear-cytosolic fractionation followed by western analysis. We also evaluated the nuclear to cytoplasmic ratio of AR-GFP by confocal microscopy comparing the relative ratios of nuclear AR in the presence of antiandrogens compared to DHT or vehicle control. Similar trends were observed by both methods although there are some notable distinctions between methods and cell types. For example, while both methods suggest that AR (or AR-GFP) are predominantly nuclear in the presence of DHT, fluorescence microscopy suggests that AR-GFP is strongly localized in the nucleus in the presence of Bic; however, when assayed by nuclear/cytosolic fractionation/western, Bic has similar levels of nuclear AR to vehicle. Similar results for Bic have been reported previously in the literature and are consistent with FRAP studies that show that nuclear to cytosolic shuttling is highly dynamic and ligand dependent and may allow some AR to escape the nucleus during fractionation.[14b, 15] Cellular localization studies with CWR22-Rv1 cells show lower levels of nuclear AR with PLM6 and PAN52 than with Bic or vehicle (DMSO).

AR-GFP fluorescence provides a snapshot of the nuclear versus cytosolic AR that may be affected by the presence of the GFP tag but avoids artifacts introduced during cellular fractionation. Studies using AR-GFP transfected CV-1 cells showed a successively decreasing fraction of nuclear AR in the order Bic > PLM1 > PLM6 > PAN52, which correlates directly with increasing ligand size (Figure 4a-f,j). Nuclear localization was the only "mechanistic" feature we found to directly correlate with the ability of our antiandrogens to evade the formation of colonies using in vitro selection assays. Whereas PLM1, PLM6 and PAN52 are all larger than current clinically used antiandrogens, molecular modeling suggests that of the four ligands tested,

PLM1 is most similar in size to the second-generation antiandrogen MDV3100 that also shares similar properties in coactivator recruitment and nuclear localization assays.[8]

In vitro selection assays cannot reproduce the complex microenvironment responsible for PCa development, expansion and metastasis found in vivo. PLM6, a compound that had distinguished itself from PLM1 and Bic in the long term in vitro selection assays and by reducing AR nuclear localization was effective in reducing C4-2B xenografts tumor weights in vivo by 49% relative to vehicle control.   Prostate cancer progression is affected by epithelial-mesenchymal interactions whichthat involve TGFβ signaling. Signaling through TGFβ associated kinase (TAK1) are required for promotion of tumorigenesis by R-Ras.[ref]   The stromal--specific inactivation of the TGF-β type II receptor blocks epithelial-mesenchymal TGF-β signaling leading to intraepithelial neoplasia in the prostate that is castrate resistant.   We found that PLM6, but not Bic, was able to increaseinduce prostate regression in tgfbr2ColKO prostates compared to vehicle control. These findings suggest that castrate-resistant cancers, previously thought impervious to antiandrogen treatmen,t may be responsive to appropriately designed antiandrogens. Even thought in vitro selections may not be able to recapitulate the complex tumor microenvironment found in vivo, compounds identified by selection in this long term format waswere effective in the tgfbr2ColKO model of "outlaw" pathway resistance.

New antiandrogens PLM1, PLM6 and PAN52 are full AR antagonists with similar potencies Bic in vitro. Long-term in vitro selections were able to distinguish between the properties of expanded arylsulphone antiandrogens PLM6 and PAN52 as compared to smaller ligands bicalutamide and PLM1. The ability of ligands to suppress the emergence of resistant clones in vitro correlated with their ability to reduce AR nuclear localization but did not correlate with down-regulation of AR or recruitment of D11 coactivator peptides in vitro. These properties also appeared to correlate with molecular size; those having structures expected to extend further into the space occupied by the agonist conformation of helix-12 were found to be more effective at reducing nuclear localization and evading resistance in vivo. PLM6 and PAN52 are effective antiandrogens in cell lines that over-express AR and those that which express truncated forms of AR that lack their C-terminal ligand-binding domains. Whereas in vitro selections cannot reproduce the complex microenvironment in vivo, PLM6 was found to reduce growth of antiandrogen and castrate resistant C4-2bB xenografts and is the first antiandrogen found to be effective in the Tgfbr2ColKO mouse model for outlaw pathway resistance demonstrating that

new AR antagonists structures can access new cellular activities not observed previously observed.

Materials and Methods:

Chemical Synthesis:

Complete details for chemical synthesis and characterization of compounds can be found in supplemental information.

In vitro growth/selection assays:

Proliferation experiments with LNCaP cells (80,000 cells/well) were plated 32 mm dishes and treated with media (phenol red-free RPMI, 5% DCC-FBS) containing appropriate amounts of antiandrogn. In vitro toxicity of compounds were determined in PC3 cells treated with 10-100 µM antiandrogen for 24 hours. Cells were fluorescently labeled using the LIVE/DEAD® Viability/Cytotoxicity Kit (Invitrogen), and quantified by microscopy as per manufacturer's protocol. Anchorage-independent growth/selection of LNCaP and CWR22-Rv1 cells was determined by the colony formation assays in soft agar, in cell suspensions of 2.5×10^4 and 1×10^5 cells.

Cellular reporter gene assays were performed using CV-1 cells transiently transfected with an androgen responsive reporter ARE-luc and wild-type or mutant

Androgen receptor action studies:

Androgen receptor reporter assays were performed in transiently transfected CV-1 cells using an ARE-luciferase reporter as previously described.[16] Western blotting was used to determine AR expression using specific antibodies for AR (N-20, Santa Cruz) and actin (Sigma). To isolate anti-androgen effects on AR degradation, experiments were performed in the presence of 44µM cycloheximide at 3 and 24 hours.

Radioligand binding assays were performed in transiently transfected COS-7 cells as described previously.[16] Coactivator peptide recruitment assays were performed by Lanthascreen LRET assay (Invitrogen, cat. No. PV4381) following manufacturers protocol. AR nuclear localization was determined by cell fractionation of LNCaP and CWR-22Rv1 cells and AR-GFP (Gift from Dr. Karen Knudsen) localization in CV-1 cells.

Molecular modeling.

Moleuclar modeling was performed using Flo/QXP on flexible models of AR and AR mutants as described previously.[16]

Mouse work (Robert/Victor/Soma).

To determine the in vivo efficacy of novel antiandrogens to elicit prostatic regression, Balb/c mice were

administered 25mg/kg·day PLM6 (Ethanol:peanut oil 1:10 v/v), PAN52 (Ethanol/Cremaphor EL 1:10 v/v) or vehicle for 3 or 5 days. Each experimental group contained 2 animals. The compounds were solubilized in ethanol prior to suspension in peanut oil or Cremaphor EL immediately prior to delivery by intraperitoneal injection. Mice were sacrificed according to UD-IACUC approved procedures. Liver, kidney, and prostate tissues were harvested, fixed in neutral buffered formalin, paraffin embedded and sectioned at 5 µm. H & E staining was performed for all tissues. TUNEL stain (Chemicon International, Temecula S7101) was performed on prostate glands according to the manufacturer's published protocol. A Zeiss Axiophot was used to take all histological images. TUNEL quantification was done by counting stained nuclei Histology was examined by a certified veterinary pathologist (EB).

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5

Dr. Ken van Golen
Madhura Joglekar Graduate Student
Yanique Conie

Determining The Sensitivity of IBC cells To Imatinib Using ATP-CRA Assay

Introduction/**Background**: Inflammatory Breast Cancer (IBC) is a rare and aggressive form of cancer. This subset of breast cancer usually represents 1-4% of breast cancers in the United Sates. Despite low incident rate, poor survival index escalates the severity of IBC disease. Clinical symptoms of IBC include primary skin changes such as erythema occupying at least one third of the breast along with peau d' orange appearance, edema, increased breast size, nipple retraction, swelling, and pain (2). This in turn causes the blockage of the blood vessels producing characteristic clinical symptoms as mentioned above (3). Due to the rarity of this disease many cases are misdiagnosed or undetected by physicians; thus, lowering the survival rate to less than 5%. IBC affects approximately 16,000 women annually, however, it is more so prevalent within the African American ethnicity. The increase within this ethnicity may be due to high body mass index (greater than 30) although, no direct correlation has been made. African American women were found to have an incidence of IBC at least 50% higher than white women (1). PDFR alpha study is relevant in IBC because of its over expression shown by our collaborators (TCRG, Belgium) in IBC patient samples. Therefore, we believe that it is important to study the signaling pathways associated with PDGFRα.
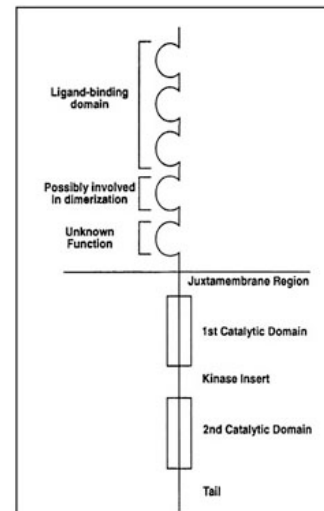
PDGF family consist of two receptors (alpha and beta) and four ligands A,B,C, and D. Combination of a specific receptor and ligand is tailored to a specific physiological role (4). PDGF ligands in dimeric form are required to bind to the receptors. For example, AA, CC,AB, and BB binds to the receptor alpha alpha. Ab, CC, and BB bind to alpha beta; and BB and DD binds to the receptor beta bata. The binding of the PDGF to its receptor induces the activity of the tyrosine kinase domain of the receptor. Once activated, the tyrosine kinase domain recruits various signaling molecules which causes appropriate response (5). PDFG signaling is inhibited by Imatinib mesylate. Imatinib binds to the ATP binding pocket present in tyrosine kinase domain; thus, inhibiting the autophosphorylation of PDGFRα and the activation of its downstream signaling molecules. The focus of this experiment is to determine the chemotherapeutic response of Imatinib using ATP based assay. In this experiment, we will be

using the ATP-CRA assay kit which is a sensitive assay that evaluates tumor cell viability by measuring the intracellular ATP levels of drug exposed cells and untreated cells (6). Intracellular ATP is the basic energy source for all living cells, and rapidly disappears when cells lose viability (7).

Significance: The significance of this assay is to find the minimum dosage necessary to sensitize the IBC cells to the chemotherapeutic drug Imatinib (Gleevec). The normal conventional therapy is exhaustive for the patients ad result in many adverse side effects . Conventional procedures begin with the diagnosis of IBC, primary chemotherapy using taxanes and anthracyclines which takes approximately 6-8 weeks. Which is followed by bi-lateral mastectomy and lymph node dissection to determine if the tumor has spread to the axillary lymph nodes. Treatment ends with high-dose, localized radiation therapy, and then further neoadjuvant theraphy (i.e. Trastuzumab if the tumor is HER2+ or Tomoxifen if it is estrogen receptor positive) is given post radiation and surgery (1). Imatinib is a direct approach towards PDGFR, as it binds specifically to the PDGF receptor and inhibits its activator and its down stream signaling (8). As opposed to the conventional procedures, Imatinib is an oral medication that has very low side effects. Further more, Imatinib has a 98 percent bioavailability. Therefore, the majority of the original dose that is administered will reach the bloodstream without being fully metabolize.

Specific Aims: To determine the EC_{50} value of Imatinib using Adenosine Triphosphate based chemotherapeutic response assay (ATP-CRA)

• To compare the efficacy of Imatinib and the combination therapy of Imatinib + standard chemotherapeutic drug based on the levels on the intracellular ATP using ATP-CRA assay

Hypothesis: Imatinib either alone or in combination with other chemotherapeutic drug sensitizes IBC cells by attenuating their viability.

Anticipated Results: We anticipate to find the effective dose of Imatinib necessary to inhibit growth and invasion of IBC cells.

• We also anticipate that Imatinib plus conventional chemotherapeutics will potentially be used for clinical procedures, and in future clinal trials.

Procedures/ Design of Experiment:

1. Approximately 2,000-20,000 IBC cells/100μl in media within low attachment 96 well plates

2. Plate enough number of wells to include treatment, no treatment, +ve and -ve control

conditions.

- Treated group: A total of 100μl drug solution (400μg Imatinib in 100μl water)
  - Untreated group: 100μl base media (100μl water)
  - Negative control: Only seeding media without cells
  - Positive control: Known amount of ATP from the ATP standard curve

3. Treated cells are incubated for 48 hours

4. Lyse cells (treated and untreated) with lysis buffer reagent

5. Add luciferace reagent solution to measure ATP levels on luminomter

6. Calculate the percentage of cell death rate.

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The expression of TRPV in prostate cancer cell lines LNCaPs and with treatment of IGF.

Talearia Young, Ben Rohe, Dr. Duncan

Significance:

In cancer patients it is still a big issue on how cancer is able to grow and metastasize. Many people who develop cancer do not die from the primary cancer, but instead die from metastatic tumor growth. Primary cancer can easily be removed from the body. The problem is cancer cells that are not seen may already have metastasized. The cancer may have metastasized to other places in the body through the blood stream. Once those cancer cells are carried to other parts, they begin to grow. In prostate cancer, one of the prominent areas of metastasis is in the bones, which may cause pain in distant organs and damage. The main problem is 1 out of every 6 males develop prostate cancer and preventing cancer from metastasizing. In 2010, there was a reported 217,730 cases of prostate cancer in the united states, and 32,050 of the men died from the cancer **(Prostate cancer, n.d.).** Most men who have metastatic prostate cancer have less then a 10% survival rate for the next five years of their lives, but other men may have a 20-30% chance depending on the location of the metastatic tumor (Brennan, J. n.d.). The significance of this research is to find out what causes prostate cancer to metastasize to bone. If successful, further research will try to prevent metastases from occurring. After removal of the primary cancer, if metastases is avoided, patients may have a higher survival rate.

Background:

 The prostate, also known as a "protector", is a tubuloaveolar exocrine gland of the male reproductive system. This gland is responsible for secreting an alkaline-milky fluid that makes up 20-30% of semen. The semen is responsible for prolonging the life of the sperm once it reaches the vaginal tract. Prostate cancer is a type of cancer that originates in the prostate of males. Most are slow growing, but there have been cases of aggressive prostate cancer. Prostate cancer cells may metastasize or travel to different areas of the body, such as the organs and even the bones. These prostate cancer cells are then able to grow in the areas that they migrate to. Three prostate cancer cell lines that are studied well, are LNCaPs, C42s, and C42Bs **(Prostate, 2011).**

LNCaP's are prostate cancer cell lines that derived from the lymph node located near the prostate and are androgen sensitive. Androgens are steroid hormones that control the development of male characteristics. This cell line was removed from the lymph node, and inserted into a mouse model. When the mouse was observed, they found that the cancer cells became androgen independent, they metastasized to other areas of the body, and they became more aggressive. Once they make their way into the blood stream and metastasize to other organs in the body, they are known as C42 cancer cell lines. These cells were then removed and put back into a mouse model. They were able to metastasize to bone, and these isolates are known as C42Bs.

TRPV, also known as transient receptor potential vanilloid, are transient receptor ion channels, which are capable of regulating osmotic pressure. Certain ones are also sensitive to temperature. These channels are specific for calcium and magnesium. Some

known inhibitors of TRP include Ruthenium red as a broad spectrum inhibitor as well as RN1734, which is known to inhibit TRPV4.

TRPV's known role in prostate cancer

According to an article titled, "Role of Cationic Channel TRPV2 in Promoting Prostate Cancer Migration and Progression to Androgen Resistance," TRVP2's expression was higher in metastatic cancer patients then in others with tumors. Previous research has shown that TRPV2 is not involved in the growth of cancer cells, but it is involved in the migration of cancer cells **(Monet, M., 2010).** It has also been shown that prostate cancer cells are able to migrate to other parts of the body via the TRPV2 channel when the prostate is stimulated by lysophospholipids. When the channel TRVP2 is activated by LPI and LPC it has been shown that the migration of cancer cells in PC3 are increased. TRVP2 has been linked to prostate cancer as playing a patho-physiological role **(Monet, M., 2009)**.

group	channel	function	tissue distribution	Ca^{2+}/Na^+ selectivity	heteromeric associated subunits	other associated proteins
1	TRPV1	vanilloid (capsaicin) receptor and noxious thermosensor (43°C)	CNS and PNS	9:1	TRPV2, TRPV3	calmodulin, PI3 kinase
	TRPV2	osmo- and noxious heat thermosensor (52°C)	CNS, spleen and lung	3:1	TRPV1	
	TRPV3	warmth sensor channel (33-39°C)	Skin, CNS and PNS	12:1	TRPV1	
	TRPV4	osmo- and warmth sensor channel (27-34°C)	CNS and internal organs	6:1		aquaporin 5, calmodulin, pacsin 3
2	TRPV5	calcium-selective TRP channel	intestine, kidney, placenta	100:1	TRPV6	annexin II / S100A10, calmodulin

IGF

IGF is a protein in humans and is encoded by the IGF1 gene. It plays an important role in childhood growth and Is referred to as a growth hormone. In most cases the liver is stimulated by a growth hormone and then secretes IGF. IGF is responsible for cellular proliferation and inhibiting cellular death **(Insulin-like-growth factor, 2011).** How does

IGF relate to prostate cancer? IGF plays a role in prostate cancer metastasis. According to an article, "Plasma insulin-like growth factor-I and prostate cancer risk: a prospective study," IGF levels in patients may be able to determine the risk of them developing cancer. Men who had a higher levels of IGF, were at a higher risk of developing cancer then men who had lower levels of IGF **(Chan, June M., 1998).**

Hypothesis:

I hypothesize that TRPV will be expressed in LNCaps C42 prostate cancer cell lines, and once the cell lines are treated with IGF or TRPV inhibitors, this will decrease metastasis f these channels in prostate cancer.

Aims of Experiment:

In this experiment, questions that will be answered are;

1. Are TRPV channels expressed in prostate cancer? If they are, is there a difference in the expression of TRP channels between each prostate cancer cell lines? Some channels may be expressed more than others. It has been shown that TRPV4 is expressed in bone cancer cell lines. Since prostate cancer cells mainly metastasize to bone, it is important to find out if TRVP4 is the link that causes metastasis to occur. We are also going to treat cells with IGF. When the cells are treated with IGF, will the TRPV levels decrease? We think this may occur because it is believed that IGF may make the cell stiff by increasing connections in the cytoskeleton, and stopping metastasis.

2. Do TRP channels regulate proliferation and migration? This will be tested by performing a scratch test on the different cell lines while treating them with TRP inhibitor to see the effects on its migration and proliferation.

This may be a breaking point in information on prostate cancer progression. This may give us information on how to stop prostate cancer cells from metastasizing to other organs or bone.

Techniques

Cell culture is a technique that will be used in the experiments. It is a process used in labs to grow cells under controlled conditions, and keeping cell lines alive outside of their original tissue source. The appropriate temperature, growth medium, and gas mixture is essential to keeping cells alive. Cell culture will be used to keep all prostate cancer cells alive and growing in this research lab.

mRNA isolation is the process of isolating mRNA to obtain DNA. This process isolates mRNA with poly tail A up to 200 adenosine to the end of the molecule. The contents of the ruptured cell will be exposed to synthetic beads where mRNA can attach. The mRNA attached to the beads are purified without washing the mRNA away. The isolation process is essential to retrieving RNA for RT-PCR.

RT-PCR is also known as Reverse Transcription polymerase chain reaction and is a technique scientists use to generate thousands or millions of copies of a particular DNA

sequence from mRNA. In PCR there are usually 20-40 repeated temperature changes or cycles. The first step is the initialization step which is when the heating reaction of 94-96 degrees Celsius (only required if there is a requirement for heat activation). The next step is denaturization, which is heating at 94-98 degrees Celsius for 20-30 seconds to cause DNA melting of DNA template. It breaks the hydrogen bonds causing a result of single-stranded DNA molecules. Other steps are annealing, extension/elongation, final elongation, and final hold **(Polymerase Chain Reaction, 2011).**

Gel Electrophoresis is used to separate biological macromolecules (DNA, RNA, Protein) on a gel. It the process of particles moving on a gel, and separating based on size and charge in an electrical field. Large molecules in the gel move slower and small molecules move faster forming bands on the gel. This process will be used in this research to see the expression of TRP's in various cell lines **(Gel electrophoresis, 2011).**

Scratch wound healing assay is sued to study the effects of experimental conditions on cell migration and proliferation. It is the process of treating with certain growth factors or inhibitors to show the effects they have on the cells growth pattern. The experimental conditions are able to be modified for different purposes. During the procedure a wound or scratch is made in a cell monolayer, and then the healing of this gap is monitored. The monitoring consists of how fast the cells grow toward the center of the gap. Growth rate, whether faster or slower than the control group, may give important information on how the factors affect cellular growth **(Chen, Y., n.d.).**

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Research Proposal

Name: Brenda Mogere

Title of Project: Effects of ATP and Adenosine on Metastatic Prostate Cancer cell line (C42-B4)

Introduction/Background/Significance of Project:

Prostate cancer is the most commonly diagnosed and second most lethal malignancy in men in USA, due mainly to lack of effective treatment for the metastatic disease. Prostate cancer cells are selectively secreted in an area of the bone marrow that is normally associated with the development and growth of cells called 'hematopoietic stem cells" (HSCs) which is the precursors of blood cells. (Article published in the Journal of Clinical Investigation). Prostate cancer cells can stay dormant in "bone marrow niche" leading to prostate cancer recurrence after a period of months or years. C42-B4 is used in this experiment. The C42-B4 is a prostate cancer cell deriving from bone metastasis. A number of studies have shown that activation of purinergic receptors attenuates proliferation of melanoma, colon and prostate cancer cells. (Article from Pub Med Neoplasia V II (II). Nov 2009.)

Previous study showed that ATP and adenosine decrease cell proliferation of both inflammatory and non-inflammatory triple negative breast cancer cell. This project will explicate effect of ATP and adenosine on metastatic prostate cancer cells and will lead to new treatment on prostate cancer.

Hypothesis

The hypothesis of the project is that ATP and adenosine will attenuate proliferation and metastatic behavior of prostate cancer cells.

Specific Aims

i) To elucidate the growth inhibitory effect of ATP and adenosine on prostate cancer C42-B4

ii) To elucidate the anti-metastatic effect of ATP and adenosine on prostate cancer cells C42-B4.

Lead Investigator/Faculty Mentor: Dr. Robert Sikes- Biological Sciences- Wolf Hall.

Brief explanation of design of experiment

After counting the cells and calculating to get the cells in the15, 000 equivalents, the cells will be treated in the 24 well plates. There will be four plates. One plate for adenosine treatment will have cells treated with half log concentrations of 3000,300,30,3, and full log concentrations of 1000,100,10,1. Each of these treatments will be in triplicates hence totaling 24 wells. The same

will be for ATP treatment.The third plate will be for control with cells grown in 6 wells. These will have no treatment on them just half change media for 7 days. The fourth plate will have Day 0 with cells in 3 wells and Background 3 wells. MTT Assay will be done on this fourth plate on Day 0. Treatment (ATP/ Adenosine) will be added directly to the wells every day after half change media. (i.e. 350 ul out and 400 ul) added for 7 days. Pictures will be taken on the first day (day 0) and on the last day (day 7).Then MTT assay will be done to get results of the number of live cells. This will be for the growth assay.

For the Scratch assay the procedure will be as follows:

1. Grow cell in 60 mm plate until they are about 95-100% confluent

2. Treat cell with ATP or adenosine at the EC50 concentration for 24 hours

3. Scratch and take pictures

4. After 24 hours, half change media and add treatment. Take picture every day.

5. Repeat step 4 for another 6 days. (total 7 days)

Anticipated results

ATP and adenosine will reduce growth rate and metastatic behavior of prostate cancer cells (C42-B4).

References.

i) Article from Pub Med Neoplasia V II (II). Nov 2009.)

ii) Article published in the Journal of Clinical Investigation.

Brezelle Bey
Lincoln University
Biology Major
John T. Koh (P.I. at UD)
"Intein based Expression and Labeling of the Androgen Receptor"

This summer I worked in the Koh Lab with John T. Koh and the Koh group, in the chemistry and

biochemistry department. I was researching the molecular biological part of prostate cancer. The title of

my project was "Intein Based Expression and Labeling of the Androgen Receptor". When I first started

my research it was slightly difficult. Simply because I am a biology major and I was working in more of an

organic based chemistry lab. Nevertheless I'm always ready to take on a challenge and willing to gain

more knowledge in any aspect of science.

Everyday I got up and went to the lab from 9am-5pm, sometimes later. A lot of the procedures I

hadn't done before so it generally took me longer than the average person. However as long as I did the

work I was supposed to do, to gain the proper results for my project I was fine with whatever work had

to be done. I had a lot of help from the graduate students in my lab whenever I really needed it, and

they also gave me a lot of insight to what research was about.

I gained so much knowledge during this internship; from information about prostate cancer on a

large aspect, the current prostate cancer treatment therapies, and how they work on a molecular level. I

don't plan to go to graduate school; nevertheless I do want to continue my research with prostate

cancer in the Koh lab as of right now, throughout my fall semester. I plan to take the MCAT next month

and hopefully get accepted to medical school. However provided that doesn't occur I would have no

problem with doing more research. It's such a stimulating thing to do, and you really do gain a lot of

knowledge. You have to have the proper background information on your work before you go into the

lab every day, but once you grasp a good understanding of what it is you're doing and how to do it, everything really does run smoothly.

I can't thank HHMI, DoD, and all those who allowed me the opportunity to engage in this internship, enough. I'm more than appreciative for it. It's a great opportunity and I would encourage anyone with a natural science major to take the opportunity because it's really worth it.